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ANTIBIOTICS

Their Chemistry and Non-Medical Uses

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Antibiotics: The..

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PREFACE

During the last ten years there has emerged a new role for antibiotics that gives promise of equaling the contribution of antibiotics in the field of medicine.

The non-medical uses of antibiotics in animal nutrition, plant disease control and food preservation are established. The first two uses are a reality, the latter promises to be as soon as further data indicating lack of public health hazard is accumulated. Thus the antibiotics can function in a vital way by extending and expanding the world's food supply.

These and other non-medical uses of antibiotics are the reasons for this volume which evaluates more than two thousand selected references in the scientific literature.

All of the contributors have extensive antibiotic research and development experience in their specialized areas and have concentrated in subject matter not generally available from any one source.

It is a pleasure to acknowledge all those who have contributed in large and small ways to this book. First thanks are due to the individual authors of the chapters for their diligent efforts which made the task of editing that much easier. Also thanks to Norman Rabjohn for reviewing the chemistry chapter and to H. D. Nauman for making available hitherto unpublished data.

Sincere appreciation is offered to the following for specific reproductions in the text: to H. B. Woodruff for Table 1-1, to E. P. Abraham and John Wiley and Sons for Bacitracin A structure, to Medical Encyclopedia Inc. for Tables 6-6, 7-3, 7-5, 7-6, 7-7, 7-8, 7-9 and to Henry Welch, W. F. McLimans, T. H. Jukes and C. G. Durbin for their permissions.

For aid in revising, rereading and reorganizing the manuscript gratitude is due my wife Helen.

HERBERT S. GOLDBERG

June 1959

Columbia, Missouri

FOREWORD

Our mid-20th-Century is often called the "Era of Antibiotics", not only because of the successful treatment of microbial diseases with antibiotic drugs, but also for many other reasons set forth in this book. That different pathways can lead toward definite objectives in the search for means of preventing and alleviating human misery is attested by successes, over the years, with anaesthetics, insulin, vitamins, sulfonamides, antibiotics, and tranquilizers. How an obscure field marked with a few observations about microbial antagonism in the 19th Century could expand into a vast area of scientific endeavor in the 20th Century deserves special comment. The flurry of antibiotics has come about, we think, chiefly for two principal reasons: (1) necessity for human conquest of microbial diseases, and (2) belief that special chemical compounds could provide means for control over these diseases. Once it was realized that metabolism, growth, antagonisms, and so on, are all cellular manifestations of underlying bio-chemical processes, then necessary steps could be taken toward well defined goals of chemotherapy. The researches of Fleming, Dubos, and Waksman showed clearly that special chemical compounds are produced in the metabolism of some microorganisms which have the striking power to inhibit growth and to destroy the life of other susceptible organisms. The application of antibiotics in the conceptual schemes of chemotherapy, in the short space of a few years, has exerted tremendous effects upon modern medical practice, the drug business, and human society.

Over the past two decades, research has brought hundreds of new chemical compounds into the realm of antibiotic knowledge. Some of these antibiotic chemicals have been applied successfully, and on a large scale, with resultant great benefits to medicine, agriculture and food processing. A few antibiotics have been used as technical aids for improving methods in the science of microbiology itself. The main applications of antibiotics, outside the field of medicine, have been in their uses as supplements in animal feeds, as sprays for crop plants and as preservatives to prevent spoilage of perishable foods. Neither the ultimate total benefits of antibiotics to mankind nor the maximum millions of dollars value in the economy of our country can be adequately foretold at the present.

For some time past, all of us have been certain about one aspect of antibiotic science, and that is the great need for a new kind of book which would summarize the remarkable advances that have taken place recently.

It is a pleasure to know that the authors of this book on chemistry and non-medical uses of antibiotics have rendered a service to all of us by bringing together factual information and interpretations of a great mass of data which have steadily accumulated. Up to now there has been no adequate summary of non-medical advances. Geneticists, biochemists, and microbiologists will profit greatly by having available the discussions on chemistry of currently used antibiotics, mechanisms of their action and their special uses in basic research. Along with fundamental knowledge about antibiotics, the detailed presentations concerning their uses in animal nutrition, for crop protection and as food preservatives will be very helpful to all who are concerned with these applied areas. Annotated bibliographies at the end of chapters give the book special value as a ready source of references.

June 1959
Brooklyn, N. Y.

PAUL R. BURKHOLDER
Research Director
Brooklyn Botanic Garden

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CHAPTER I

INTRODUCTION

BY H. S. GOLDBERG AND T. D. LUCKEY

O wad some Pow'r the giftie gie us
To see oursels as a bacillus.
It wad frae mony a blunder free us,
And follish notion.

—Burns, with variation

A. HISTORY AND DEFINITIONS

The origin of the term "antibiotic" appears to have been in some dispute. Burkholder, in an excellent article on the mutual relationships of microorganisms, traces the origin of the term back to 1889.¹ However, disputing the point, Waksman lays claim to his own introduction of the word into the literature in the early 1940's.² Florey *et al*³ would seem to agree with Waksman, as they report that he (Waksman) proposed a definition in 1945. Burkholder⁴ has rebutted this view, and quotes a passage in French written in 1928 that appears to use the word "antibiotic" in discussing the phenomenon of antibiosis.

Further searching indicates Vullemain in 1889^{5a} first applied the term antibiosis to an often-observed phenomenon of antagonism between living organisms. The word antibiotic soon became part of the description. It had, therefore, existed in print prior to the 1940's, and it was defined in the dictionary as "pertaining to antibiosis, tending to destroy life."⁵ However, insofar as antibiotics are now related to microorganisms, it seems to be true that the word in its *present restrictive meaning* was used and interpreted originally by Waksman.

The most adequate definition for antibiotics as they are discussed in this volume is "chemical compounds derived from microorganisms which have the capacity of inhibiting the growth of, and even destroying other microorganisms". This definition originated in the literature in 1942.⁶ Some time later Benedict and Langlykke⁷ added the specification that these compounds be effective in low concentrations, and Waksman has since modified his original definition by adding the words "in dilute solutions" to the definition above.²

Other definitions have found favor at various times in order to make it possible to include those antimicrobial compounds that are synthesized

from higher plants and animals. Thus in Langlykke and Benedict's definition antibiotics come from living organisms, not necessarily microbes. If this meaning is followed, the number of antibiotics reported in the literature approaches 3-4 thousand. Following Waksman's definition we find approximately 300 well-documented antibiotics produced by microorganisms.⁸

The present discussion is restricted to those antibiotics that fit Waksman's definition, that are commercially available and that have non-medical functions; as well as those that contribute, by their chemical and biological properties, to the purpose of this volume.

In 1877 Pasteur and Joubert⁹ noted that aerobic bacteria antagonized growth of *Bacillus anthracis*. Furthermore, they noticed that experimental anthrax in susceptible animals could be repressed by simultaneous inoculation of various non-pathogenic bacteria. Pasteur noted that this phenomenon of interaction between microbial species might ultimately find therapeutic uses.

Babes¹⁰ in 1885 was the first to interpret his own experiments on microbial antibiosis as being due to production of an inhibitory chemical substance by the antagonistic organisms.

Nine or ten years after Babes' work, Emmerich and Low¹¹ prepared an extract of *Pseudomonas pyocyaneus* which they called pyocyanase. In very high dilution this compound was shown to be inhibitory to organisms of diphtheria, typhoid and plague, as well as to pathogenic cocci. The Emmerich and Low report resulted in an intensive study of the use of this compound in treating human disease.

For approximately twenty years, pyocyanase was used by clinicians in treating a variety of infectious diseases. These therapeutic attempts are documented by Florey *et al.*,³ and provide enlightening reading which establishes the fact that a thorough investigation was conducted in antibiotic therapy many years before the current "Antibiotic Era." Unfortunately pyocyanase, although effective, was toxic, and therefore confusion and misunderstanding resulted to a marked degree before the work was abandoned.

Some years later other bacteria were shown to have similar inhibitory effects on pseudomonas. Nicolle in 1907¹² gave one of the earliest reports of antibacterial action for *Bacillus subtilis*. Since then many reports have appeared concerning the antibiotic activity of spore-forming bacilli.^{13,14,15}

In a series of studies, Gratia and Dath^{16,17} extracted a lytic agent from a mold and used it in successful treatment of staphylococcal skin infections. These authors were among the first to begin a systematic search for antagonistic organisms from natural sources. This work

progressed rapidly among soil and plant microbiologists of the day, and reports emerged confirming antagonism by fungi. The occurrence of antagonistic properties of molds thereby became an established fact, culminating in the discovery of penicillin by Fleming in 1929.¹⁸

Fleming's report on the antibacterial activity of his mold-contaminant is notable for the clarity, completeness and detail in which the antibiotic activity of penicillin is described. Surely, without such a detailed analytical report the penicillin development work would never have followed.

Beginning in 1938 Florey and Chain,¹⁹ and Abraham and associates²⁰ reevaluated the therapeutic possibilities of penicillin as suggested by Fleming. These studies were carried out at the William Dunn School of Pathology, Oxford University.

In 1941, Florey and Heatley were invited to the United States to hold conferences with personnel of the U. S. Department of Agriculture, of the Committee on Medical Research of the Office of Scientific Research and Development, and of the National Research Council. As a result of the conferences, private industry became interested in the commercial production of penicillin.

The rapid and extensive expansion of the penicillin industry was based on the cooperative planning and the vast amount of research of government agencies, universities, industrial organizations and medical facilities in the United States and Great Britain.

During this time Dubos,²¹ at the Rockefeller Institute for Medical Research, capitalized on the observation that certain pathogenic organisms disappeared from infected soils. By careful and well-planned study, Dubos obtained the antibiotic tyrothricin from the soil bacillus, *Bacillus brevis*. Although tyrothricin now has limited use, Dubos contributed immeasurably toward forcing a realization of the potentialities of antibiotic substances. The work of Dubos and associates on the study of the chemical, biological and physical properties of this antibiotic was a great contribution to what may be considered to be the beginning of the "Antibiotic Era."^{22,23}

Immediately following the work of Dubos, a report emerged describing the first antibiotic to be obtained from a culture of an actinomycete. This antibiotic was called actinomycin, and was discovered by Waksman and Woodruff.²⁴ Waksman and his co-worker's contributions continued along these lines and eventually resulted in the first actinomycetes-produced antibiotic which found clinical application, streptomycin.²⁵

The impact of the discovery of streptomycin on the search for antibiotics from actinomycetes was great and soon more than 15,000 actinomycete strains had been examined.^{26,27} As a result of these and more

recent screening programs, a considerable number of antibiotics have been made available.

In 1945 bacitracin was discovered,²⁸ in 1947 polymyxin³⁰ was reported the same year that chloramphenicol was reported by Ehrlich *et al*,²⁹ in 1948 chlortetracycline was found,³¹ in 1949 Waksman contributed neomycin³², and in 1950 the Pfizer group found oxytetracycline.³³ In 1952 erythromycin³⁴ and carbomycin³⁵ were reported.

Since 1952 the pace has slackened somewhat. More specific aims have characterized screening programs. The search continues, but instead of seeking only antibacterial agents, efforts are made to find antifungal agents, antitumor compounds, antiviral agents and similar materials.

TABLE 1-1
NEWLY-NAMED ANTIBIOTICS

Antibiot. Ann. 1953-4	J. Antibiot. (Japan) 1953	
Hygromycin	Azomycin	Pyridomycin
Methylmycin	Flaviciid	Sarcidin
Ruticin	Flaveolin	Sarkomycin
Streptocardin	Griseoflavin	Achromoviromycin
Streptogramin	Leucomycin	
Tetracycline	Pthiomycin	
1954-5		1954
Actinomycin III	Actinoleukin	Fermicidin
Celesticetin	Albomycetin	Mediocidin
Etamycin	Angustmycin	Nocardorubin
Oleandomycin	Aureothin	Seligocidin
Pleomycin	Brevolin	Zaomycin
Spiramycin	Eumycetin	Carzinophilin
1955-6		1955
Amphotericin A and B	Amaromycin	Grasseriomycin
Cathomycin	Cereviocidin	
Eulicin	Grisamine	
Synergistin	Mesenterin	
Rubidin	Ractinomycin	
Streptolydigin	Tertiomycin	
Thiostrepton	Thiomycin	
Vancomycin	Violacetin	
1956-7		1956
Alazopeptin	Carzinocidin	Mitomycin
Nucleocidin	Pluramycin	Phagomycin
Ristocetin A and B	Ganicidin	Phagocidin
	Mikamycin	Phleomycin
		Toyocamycin

* Woodruff, H. B. "Strategy of Chemotherapy" Cambridge Univ. Press 1958.

It is in the above areas that the future screening programs are likely to concentrate in order that the current non-susceptible microbial agents may be brought under control.

The problem of bacterial resistance to antibiotics has evolved, and antibiotics are sought for use against those organisms which rapidly develop resistance, or which are naturally refractory. Thus, there are now antibiotics which are specifically recommended for use against penicillin-resistant staphylococci, for *Proteus* and *Pseudomonas* sp., for streptomycin-resistant tubercle bacilli, etc.

The newest agents such as cathomycin,³⁶ oleandomycin,³⁷ fungicidin,³⁸ ristocetin,³⁹ vancomycin,⁴⁰ kanamycin⁴¹ and several others are destined to have a significant role to play in microbial inhibition. Undoubtedly these antibiotics will also be investigated for their non-medical uses and so find applications in that field, following the course of the earlier work.

Table 1-1, adapted from a recent contribution by *Woodruff,⁴² illustrates some of the antibiotics reported since 1953.

B. ECONOMIC IMPACT

The impact of antibiotics on the economic structure of the pharmaceutical industry has been immense; and its effect has extended more recently to the feed business.

Table 1-2 gives a growth study of antibiotic production for an eleven-year period. Examination of these figures shows that, in 1943, the first commercial year of penicillin production, a pound of this antibiotic was worth more than one hundred thousand dollars! In eleven years the price came down to less than seventy-five dollars per pound and eight hundred sixty thousand pounds were produced.

Streptomycin, the next important antibiotic to become available in commercial quantities, is currently being produced at the rate of half a million pounds annually.

The broad spectrum antibiotics (the tetracyclines and chloramphenicol) achieved an annual production valued at one hundred and thirty seven million dollars within five years after the first such antibiotic was reported in 1948.

Then there is the application of antibiotics in feed supplements, essentially a non-medical use. This use of antibiotics was first recognized in 1950. One year later more than two thousand pounds of antibiotics went into animal nutrition.

In 1954 more than 20 per cent of all antibiotics produced in the United States was used in feed supplements. Considering this outlet, in addi-

TABLE 1-2
ANTIBIOTIC PRODUCTION IN THE UNITED STATES
1943-1954

Year	Antibiotic	Pounds Produced	Value in Millions of Dollars
1943	Penicillin	29	3
1946	Streptomycin	3,800	11
1951	Penicillin	636,000	137
1951	Feed Supplement Antibiotics	236,000	17
1952	Feed Supplement Antibiotics	258,000	17
1953	Penicillin	756,000	58
1953	Broad Spectrum Antibiotics	417,000	137
1953	Streptomycin	375,000	35
1953	Feed Supplement Antibiotics	434,000	19
1954	Penicillin	860,000	63
1954	Streptomycin	494,000	40
1954	Broad Spectrum Antibiotics	440,000	150
1954	Feed Supplement Antibiotics	479,000	25
1954	All Antibiotics	2,284,000	272

tion to the present and potential uses of antibiotics in plant disease control, food preservation, etc., the figures for the next eleven years should show an equally impressive advance.

C. NATURAL OCCURRENCE OF ANTIBIOTICS

1. KINDS OF ORGANISMS PRODUCING ANTIBIOTICS

As mentioned above, more than 15,000 strains of actinomycetes have been examined for antibiotic properties. Of these, more than 2,000 have shown some antibacterial effect. This figure indicates that the number of microorganisms producing antibiotics must be very large indeed. An interesting question arises as to how these antibiotic-producing organisms are concentrated (in the soil), and what happens to the antibiotics produced in the soil under natural conditions.

Soil microflora are controlled, as is any mass population, by the available food supply. Thus one can readily anticipate fluctuations in overall population as organic matter is deposited and digested in the soil. In spite of this variable, however, it is possible to ascertain specific facts relative to kinds of antibiotic-producing microbes in the soil.

The actinomycetes are the most common antibiotic-producing microorganisms found in soil. They have been studied the most, are biologi-

cally the most active, and to date they have produced the greatest number of commercial antibiotics.

Schatz & Hagen⁴³ and Colligan⁴⁴ and others have noticed that actinomycetes as a group contain a greater proportion of activity than fungi or bacteria. In a comprehensive study on soil antagonisms, Strossel, Leber & Keitt⁴⁵ found that 80% of antagonism to fungi was due to actinomycetes.

The bacteria of the soil, on the other hand, appear to be relatively quiescent as regards noticeable antagonism under natural conditions. Generally the bacteria require increased nitrogen, organic matter and related substances before initiating antagonism.⁴⁶ In spite of the fact that the soil has contributed at least two notable bacteria-produced antibiotics, tyrothricin and polymyxin, the bacteria are not expected to contribute much to the future of the soil antibiotic picture.⁴⁷

Comparing soil fungi with soil bacteria shows that fungi are much more active as microbial antagonists.⁴⁷ Jeffreys⁴⁸ found that of 65 species isolated from soil, about one-half produced antibiotics. Luke and Cornell⁴⁹ found that about 16% of the fungi, and 3.6% of the bacteria isolated from sugar cane soils were antagonistic.

The facts of microbial antagonism in soils and its relation to potential antibiotic production indicates that the actinomycetes will continue to be the most important source, followed by the fungi and the bacteria.

2. ANTIBIOTIC PRODUCTION IN NATURE

Since many cases are known of antagonism between microbes in their natural habitats, antibiotic action is usually assumed to be the mechanism. However, this view is subject to some question, in view of the fact that commercial antibiotic production requires pure cultures and highly enriched media, whereas natural soil always offers mixed cultures, and frequently contains depleted nutrients.

The present direct evidence of the production and persistence of antibiotics in the soil is based on experiments performed in sterile supplemented soil, in unsupplemented soil, and in soil with normal microflora. Available data indicate that antibiotics are inactivated in soil by adsorption (the basic antibiotics), by instability of the pH of the soil, by chemical reaction with a soil component, and by biological attack.^{50, 51, 52}

As evidence of these mechanisms, Brian in 1949⁵³ showed that persistence of gliotoxin in soil was dependent on pH, Pramer and Starkey⁵⁴ found that streptomycin was susceptible to pseudomonas attack in

TABLE 1-3
ANTIBIOTICS DEGRADED MICROBIOLOGICALLY IN SOIL

Actinomycin
Albidin
Chloramphenicol
Chlortetracycline
Cycloheximide
Frequentin
Globisporin
Griseofulvin
Mycophenolic acid
Oxytetracycline
Patulin
Penicillin
Streptomycin

soil and Siminoff & Gottlieb⁵⁵ showed that streptomycin was inactivated by soil colloids. Many other workers have reported various similar conditions under which antibiotics are adsorbed by clay minerals and organic materials in the soil.^{56,57,58}

TABLE 1-4
ANTIBIOTIC PRODUCTION IN

Organism	Soil (normal flora)	Sterile Soil	Sterile Soil (plus supplement)
Fungi:			
<i>Aspergillus clavatus</i>	NT	0	patulin +
<i>Aspergillus terreus</i>	NT	0	citrinin +
<i>Penicillium nigricans</i>	0	0	griseofulvin
<i>Penicillium frequentans</i>	frequentin	NT	NT
<i>Penicillium patulin</i>	patulin +	patulin +	patulin +
<i>Penicillium gladioli</i>	gladiolic acid	NT	NT
<i>Trichoderma viride</i>	gliotoxin	gliotoxin	gliotoxin
<i>Trichothecium roseum</i>	NT	trichothecin +	trichothecin +
Actinomycetes:			
<i>Streptomyces antibioticus</i>	NT	actinomycin	actinomycin +
<i>Streptomyces griseus</i>	0	0	cycloheximide +
<i>Streptomyces venezuelae</i>	0	chloramphenicol	chloramphenicol
<i>Streptomyces</i> spp.	0	confirmed	actinomycin
<i>Streptomyces</i> spp. A67, B, 287, and B	confirmed	0	confirmed
Bacteria:			
<i>Bacillus</i> sp. B6	confirmed	0	confirmed
<i>Pseudomonas fluorescens</i>	NT	NT	confirmed

Note:

+ denotes the compound was not fully identified.

0 shows no antibiotic was found.

NT not tested.

Further evidence that antibiotics are attacked biologically in the soil is abundant. Two recent reviews by Pramer⁵⁹ and by Brian⁶⁸ establish that at least a dozen antibiotics are microbiologically degraded in soil. It is not unreasonable to expect that under certain conditions all antibiotics are susceptible to such degradation by soil microflora. Table 1-3 lists these antibiotics which have been reported to be inactivated much more rapidly in non-sterilized soil than in sterile soil.

Brian has stressed the difficulty of establishing an antibiotic organism in the soil, and of insuring conditions for adequate antibiotic production. Nevertheless in several instances *in vitro* production of antibiotics in soil has been recorded. The production of griseofulvin, gliotoxin, chloramphenicol and actinomycin has been proved by specific microbiological assay, or other methods of accurate identification. In other instances indirect evidence has been accumulated. Table 1-4, which has been adapted from Brian's most recent work,⁵⁸ illustrates these successful studies.

Thornton and Meilkejohn⁴⁷ have injected a note of caution to be applied to all results on antibiotic detection in the soil. Failure to detect antibiotics in soil cultures of organisms known to be capable of their production may be due to a lack of proper technique. Stevenson⁶⁰ has used successfully a method other than soil extraction. Ehrlich *et al.*,⁶¹ in testing recoverability of chloramphenicol from soil extracts, reported that 40 per cent of the amount added to sterilized soil could be recovered in an experiment of three months duration. In natural soil, however, 45 days was the maximum limit for detection at any level. All this work was based on the initial introduction of a level of 4.6 mcg of antibiotic per gram of soil.

3. BIOLOGICAL SIGNIFICANCE OF ANTIBIOTICS IN SOIL

In considering the biological influence of antibiotics in soil, one must deal both with those antibiotics produced naturally in the soil, and also with those which find their way to soil following antibiotic treatment of crops and other plants for disease control. How do these antibiotics affect such properties as soil fertility, seed germination and plant growth? Is it possible to induce biological control of the soil with antibiotics?

Unfortunately, these two questions appear to be unanswerable at this time. Limited evidence from soil investigations indicates the existence of some effects on microbiological soil processes, on seed germination and on plant growth, but the necessary large-scale studies have yet to be performed.

Studies on the effects of antibiotics on nitrogen-fixing bacteria and

nitrifying bacteria have been made by Waksman and Woodruff⁶² and by Pramer and Starkey.⁶³ However, this work was usually limited to laboratory experiments which may not be directly applicable to the soil.

In actual soil studies, oxytetracycline⁶⁴, streptomycin⁶⁵ and chloramphenicol⁶⁵ inhibited nitrification in the soil. In an interesting report, Nissen⁶⁶ indicated increased total flora and increased CO₂ production in soil to which specific antibiotics had been added.

Effects of soil antibiotics on higher plants have been noted frequently. Phytotoxic action seems to occur with certain of the antibiotics. Adverse effects on shoot and root growth, on chlorophyll production and on germination of seeds have been noted.^{67,68} A severe bleaching effect by streptomycin on algae and on seedling chlorophyll has been reported by several workers^{69,70} (see also Chapter 4). The mechanism of this action is not known.

Of a more controversial nature is the data on the effect of soil antibiotics in stimulating plant growth.⁷¹ This work has yet to be confirmed. If the antibiotics in proper concentration can stimulate growth of plants regularly under field conditions, the benefits would be overwhelming (See Chapter 3.)

Actual trial of antibiotics for the biological control of root disease has had some positive success. The work in this field has been reviewed recently by Thornton and Meilkejohn,⁴⁷ and by Wood and Tveit.⁷² An additional survey of the problem is presented by Pramer.⁵⁹

In conclusion the evidence indicates that some antibiotics are produced in natural soil, and that other antibiotics get into the soil from agricultural dusts and sprays. However, the mechanisms by which soil and its components affect the antibiotic activity is not established. It is therefore difficult to predict the eventual harmful or beneficial results that these antibiotics might induce.

If one believes, as does Brian,⁵⁸ that antibiotics play a role in evolution of soil microflora within micro-environments, the antibiotics are beneficial, at least to the soil microflora.

D. EFFECTS OF ANTIBIOTICS

1. EFFECTS OF ANTIBIOTICS ON THE ORGANISMS PRODUCING THEM

By the definition given earlier in this chapter, an antimicrobial compound must be a special inhibitory product of a microorganism or a substance that is very similar to such a compound. (This definition excludes lactic acid, ethanol or proteolytic enzymes which are a part of the everyday products of microbial metabolism.) Axiomatically,

the organism producing an antibiotic is resistant to its action, and such organisms are logically to be sought in media in which the antibiotic is being produced. Umezawa *et al*⁸⁹ found many chloramphenicol-producing (and resistant) strains by growing organisms in media containing chloramphenicol. How the organism remains immune to its own "poison" is explained in part later in this chapter.

Few studies give direct evidence of the effects of antibiotics upon the organism which produces them. The environmental conditions and genetic considerations required to produce maximum quantities in commercial fermentation have been considered in detail by others. While it is known that generally conditions favorable to the growth of the organism also favor antibiotic production, interesting questions about the organism remain unanswered. Why do two strains of antibiotic-producing organisms differ in their production capacities? Does the presence of the antibiotic produced cybernetically cut off or reduce the antibiotic production-line of the cell? Does the presence of a minute quantity of an antibiotic from another species stimulate early maturation of the antibiotic production machinery of a cell?

Presumably the production of antibiotics by a cell is useful to the organism as a survival mechanism rather than as an incident of metabolism. However, even though all soil microorganisms are probably concerned with some phase of antibiosis (up to $\frac{1}{2}$ of the actinomycetes that have been isolated produce one or more antibiotics), the production of antibiotics in soil does not allow one strain to maintain the "balance of power" to the exclusion of other organisms. Stevenson¹⁵⁸ showed that the same changes are seen in sensitive test organisms when exposed in soil either to crystalline actinomycin or to a culture of *Streptomyces antibioticus* which is known to produce actinomycin. This and the other evidence discussed above indicates that antibiotics are produced in the soil. Antibiotics may be defense mechanisms used to establish territorial rights rather than offensive weapons used to extend the limits of ownership.

Strangers in the land of soil microorganisms have little chance for survival: conditions and competition are so harsh that soil is not a reservoir for pathogens, but it is a true burial ground for disease—delicate parasites cannot survive in the company of soil microbes.

Does the antibiotic in the soil really help its producer? Brian⁵⁸ reviews the available evidence. When microorganisms are isolated from fragments of organic matter in the soil, a high proportion produce antibiotics. Antibiotic-producing strains are more effective antagonists to root pathogens, in soil, than other strains of the same species which do not produce antibiotics. Inoculation of antibiotic-producing sapro-

phytes along with root parasites into sterile soil has reduced the vigor or prevented the survival of the parasite. Unfavorable soil conditions decrease the antagonistic activity of antibiotic-producing organisms. While other experiments do not support this thesis, there seems to be enough data to warrant continuation of work such as the inoculation of seeds with antagonist organisms. Wright¹¹² could extract frequentin, gladiolic acid or gliotoxin in the mustard, pea, or wheat seeds he had inoculated with fungi and placed in soil. In human fecal residues, antibiotic producers occasionally "take over" for short periods of time. The transient nature of this phenomenon is not understood.¹⁴⁵ Burkholder and Burkholder suggest that antibiotic production may be a factor of symbiosis in lichens and corals.²⁰⁷

Consider the production of antibiotics from the point of view of the microbe. Evidently few organisms produce detectable quantities of antibiotics in "average soil". Average soil is nutritionally exhausted as far as maximum bacterial activity is concerned. Microbial populations have exhausted the most common limiting nutrient (usually a carbon energy source) in an intense flurry of metabolism, growth and reproduction while a good supply of material was available. The myriad microhunters then entrench until the next bonanza—a cow defecates, a worm dies, a farmer plows under the straw from the wheat harvest—each event produces a welter of activity from soil microorganisms. Now, if ever, is the time and the place for microbes to use antibiotics in the struggle for existence. Those with the greatest striking power will leave the most progeny. As nutrient resources are used up in each microvillage (a tablespoonful of rich soil contains more microorganisms than there are people on the earth today) the population again becomes quiescent.

2. EFFECT OF ANTIBIOTICS UPON OTHER MICROORGANISMS

In the consideration of the effect of antibiotics upon other microorganisms, a logical first step is to simplify the concept of "other microorganisms" from that of all known species to the vastly smaller number of those which react in a given way to a particular antibiotic. Some organisms are susceptible to the antibiotic, some are resistant, some develop resistance and some may be nutritionally dependent upon it for their growth. We shall return to a consideration of each of these states.

The very definition of antibiotics demands that this chemically heterogeneous group of compounds be considered as one group because they are antagonistic to microorganisms. Consideration of the chemistry of their widely diverse molecular structures (see Chapter 2) indicates

they are similar only in their common microbial origin and action. Hence, it has seemed reasonable to attempt to classify antibiotics primarily upon the basis of their antimicrobial spectrum. Chemical and physical characteristics are thus of secondary importance: although in the compounds related closely by structure the biological characteristics of one compound resemble those of the others (they are said to be of one chemical family). Although many of the broad-spectrum antibiotics are effective against rickettsia and large viruses, none seem to be specific for this group or organisms, and, more unfortunately, no antibiotics are known to be efficient antiviral substances for the small viruses. Future research should help to fill these blanks.

The effect of adding an antibiotic to a culture of organisms may be (1) destruction of cells; (2) depression of reproduction, growth, respiration, or metabolic rates; (3) change leading to increased resistance of the cell; (4) alteration in physical and chemical properties and reactions of the cell in innocuous pattern; (5) no apparent effect; (6) a decrease in competitive organisms (in mixed culture) to allow more growth; (7) a direct growth and/or metabolic stimulation; (8) establishment of nutritional dependency; and (9) a combination of several of these effects. Sensitization does not seem to occur in bacteria.

The use of antibiotics in the past decade has led to the appearance of an ever increasing number of resistant strains—the relative specificity of the antibiotics have challenged the potential for evolution of microorganisms in many climes. Many of the “side reactions” of antibiotics make them most useful tools in biology. Chloramphenicol is found to increase the lysogenic reaction in bacteria subjected to temperate virus,¹⁹⁵ and as they may be mutagenic agents²¹³ it is possible that they will be found to induce lysis in lysogenic bacteria.

a. Susceptible Organisms

We are not satisfied simply to say: this antibiotic inhibits these organisms. We want to know how—what are the mechanisms which determine the specificity of a compound? This is the major topic that is examined throughout the rest of this chapter.

Concentration is an important factor. At low levels the antibiotic has no effect, or may actually stimulate growth of susceptible organisms. This stimulating action is considered in the chapter on nutrition. When the concentration is raised to cytotoxic levels, there is a direct relationship between the concentration of antibiotic in the medium and the antimicrobial effects, until a level of maximum effectiveness is reached. Many antibiotics are bacteriostatic in low concentrations and bactericidal

in higher concentrations. When a dose above the optimal concentration is used, the antimicrobial effect is decreased: this is the "paradoxical zone phenomenon".¹⁵⁹

Susceptibility of organisms to an antibiotic constitutes the antimicrobial spectrum of that compound. The antimicrobial activity of representative, major present-day antibiotics is given in Table 1-5.

TABLE 1-5
ANTIMICROBIAL SPECTRUM OF SELECTED ANTIBIOTICS^a

Antibiotic Organisms		Tetra- cyclines			Marco- lide			Poly- ene		Polypeptide				Miscellaneous				
		Tetracycline	Oxytetracycline	Chlortetracycline	Erythromycin	Carbomycin	Fumagillin	Nystatin	Rimocidin	Tyrothricin	Polymyxins	Bacitracins	Neomycin	Penicillins	Chloramphenicol	Novobiocin	Streptomycin	Cycloheximide
Virus (large)		2	2	2	1	2	1	0		0	0	1	0	1	2	0	0	
Rickettsia		2	3	3	2	3		0		0	0	0	0	0	3	0	0	
Bacteria:																		
gram +		3	3	3	3	3	1	0		3	0	3	2	3	2	3	2	0
gram -		3	3	3	1	1	0	0		1	3	1	3	1	3	0	3	0
acid fast		1	1	1	1	0	0	0		0	1		2	1	1	0	3	0
Fungi:																		
Plant pathogens		0	0	0	0	0	0	3	2	1	0	0	0	0	0		0	2
Animal pathogens		0	0	0	0	0	0	3	3	1	0	0	0	0	0		0	1
Saprophytes		0	0	0	0	0	0	3	2	1	0	0	0	0	0		0	2
Yeasts		0	0	0	0	1	0	3	1	1	0	0	0	0	0	0	0	3
Protozoa		0	1	1	2	2	2	0	1	2	0	0	0	0	0	0		1

Key: 0. Generally Ineffective.

1. Effective in a few representatives.

2. Effective *in vivo* for several representatives.

3. Most effective.

Since penicillin is effective primarily against gram-positive organisms, it is called a narrow spectrum antibiotic. A broad spectrum antibiotic such as chloramphenicol or oxytetracycline is effective against a wide variety of organisms. The data indicate that chemically related compounds have a similar spectrum, if they are active at all. It is also noted that compounds effective against the fungi have little activity against other organisms and *vice versa*. We may thus suspect the existence of

at least two very different general phenomena in the mechanism of action of these two major groups of compounds. A review of the specific actions of antibiotics indicates that one differentiating mechanism may be the structure (physical and chemical) of the cell wall, and the permeability or binding of the antibiotic to the various components of the wall.

b. Resistant Organisms

After inquiry into the mechanism by which cells are susceptible to an antibiotic, it is almost redundant to ask why some organisms are resistant to the drug. A complete explanation of the susceptibility of either, at the molecular level, necessarily involves the other. One of the variables in susceptibility is the number of organisms present: the effectiveness of an antibiotic decreases at a rate corresponding roughly to the logarithm of the concentration of organisms. Welch¹⁰⁹ illustrates the variability to be expected in a microbial population from his studies of streptomycin resistance to a variety of microorganisms:

Streptomycin level (mcg/ml.)

0.05– 0.2

0.2 – 10

10 –1000

Survival of organisms

Equivalent to Control

Rapid Increase of Death Rate

One Survivor per 10⁸ Cells

These data indicate that about 10 organisms in one billion have an inherent resistance to this drug (translated into population figures this would mean that about 30 people would survive a similar catastrophe applied to our world if people had heterogeneity equivalent to that of the organisms studied). Many of these survivors are totally resistant to high concentrations of the drug, and they transmit this characteristic to their offspring through many generations in the complete absence of streptomycin. The replica plating technique of Lederberg indicates that the resistant organisms were a part of the original population.¹⁸⁰

The exact mechanism of variation in natural resistance may not be well understood until the specific action of the antibiotic is known. In some cases (i.e. the increased production of penicillinase by penicillin-resistant organisms in the presence of penicillin) natural resistance can be increased by incubating the organism with sub-lethal levels of the antibiotic. This effect may be due to adaptive enzyme formation.

Resistant organisms may use a variety of actions to remain immune to the action of an antibiotic. The antibiotic may be unabsorbed, destroyed by an enzyme (e.g. penicillinase is sometimes found as an extracellular enzyme and sometimes as an intracellular enzyme), inactivated by binding it to a protein or mineral, metabolized or detoxified,

as is chloramphenicol,¹⁰⁰ within the cell. Finally, the antibiotic may be ineffective because the metabolic reaction which it blocks may be absent in the resistant organism. Thus penicillin may interfere with glutamic acid absorption in susceptible organisms, while penicillin-resistant organisms can synthesize glutamic acid.¹⁰⁷ Streptomycin resistant variants of *E. coli* do not appear to use the same terminal respiration cycle as does the streptomycin-sensitive parent strain. Thus the oxaloacetate-pyruvate condensing reaction which is specifically inhibited by streptomycin is not used in some resistant or dependent strains.¹²²

c. Acquired Resistance

The resistance discussed previously is found in organisms which have inherited a resistant metabolic state prior to contact with the drug. The most important problem arising from indiscriminate use of antibiotics is the development of resistant strains. Jawetz¹⁴¹ reviews the reviews on this subject. Acquired resistance presents exciting possibilities to examine the mechanisms by which microorganisms adapt to new environments. This adaptation may be acquired by different mechanisms which are very difficult to differentiate. When an antibiotic blocks the usual pathway of metabolism, a single organism is considered resistant if it metabolized by an alternate pathway; or an organism can acquire resistance to the drug by (1) conditioning itself to absorb less of the drug; (2) by developing enzyme(s) to destroy or inactivate the compound; (3) or by developing an enzyme system to metabolize by an alternate pathway. In either case, the adaptation may be accomplished by means of (1) adaptive enzymes—increasing the quantity of an already existing enzyme; or (2) by a mutation which might provide the needed enzyme potential where none had existed before; or (3) simply by resistant individuals surviving to reproduce more resistant organisms to displace sensitive organisms of the culture in the antibiotic medium. In each of these cases the resulting population grows from cells which were initially, or have become, more resistant—the culture may acquire resistance through these different mechanisms. If the organisms were not already genotypically resistant, as under (1), the continued growth in the presence of antibiotic is favorable to the development of genotypic resistance.¹⁷³

Cavalli¹¹¹ points out that the development of drug resistance does not appear to act as an adaptive enzyme mechanism, because enzyme adaptation occurs in a large proportion of cells and is reversible in the absence of the stimulating substrate. He then gives evidence to indicate that neither of these conditions apply, thereby indicating the genetic char-

acter of this resistance. He suggests that streptomycin-induced resistance may follow a one-locus (or one gene) all-or-none resistance (although it is sometimes stepwise)¹⁸³; while the stepwise pattern of resistance which organisms develop to penicillin, chloramphenicol and oxytetracycline indicates polygenic participation.

Akiba¹⁷² suggests that streptomycin induces mutation to give resistant strains—bacteria grown on sub-inhibitory levels become highly resistant. Simultaneous multiple chemotherapy with synergistic antibiotics may be developed as methods are developed to use it effectively to decrease the number of successful mutations.

Bryson and Demerec¹⁸³ suggest that resistance is primarily a genetic phenomenon, usually involving many steps. The culture adapts to a low level of antibiotic, with some of the population resistant to greater concentrations than that in which any of the organisms could previously survive. This process is repeated several times, and the culture is soon immune to very high levels of the antibiotic. The reference cited reviews the evidence for genetic recombination as a factor in resistance.

Evidence for the genetic basis of the inheritance of resistance is obtained by the induction of resistance to sensitive organisms by adding isolated DNA from resistant organisms to the culture of the sensitive organisms.^{185,184} This transforming agent can be found only in resistant organisms. Zinder and Lederberg¹⁸⁶ report similar results by transduction—the transfer of resistance-inducing genetic material via bacteriophage from a resistant organism to a sensitive organism.

Hinshelwood¹⁸⁷ suggests that even when clear-cut evidence for genetic mutation exists, adaptive enzymes are still playing their part in the resistance symphony. His best argument for this position is that newly-acquired changes may be quickly lost in the absence of the drug, while long-held characteristics are very stably held. One would suggest that the longer adaptive enzymes allow an organism to survive in the presence of an antibiotic, the greater is the probability that a genetic adaptation could occur to give genotypic permanency to the changed phenotype. Northrop finds the occurrence of oxytetracycline-resistant organisms corresponded to the number expected by calculation of mutation rates.²⁰¹ Christensen²¹³ suggests that antibiotics are mutagenic.

Eagle and Saz¹³² summarize the controversial evidence of acquired resistance. It is not possible at the present time to interpret these data exclusively in favor of either of the two hypotheses—that is, either that the change is genetic or that the phenomenon of acquired resistance is merely the survival of those organisms with more “natural resistance.” Both effects are probably at work, since they are not mutually exclusive. Induced resistance appears to be less permanent than natural resistance.

Organisms acquire resistance to penicillin, as mentioned above, by many small increments and not by one single large step.⁸⁵ Acquired resistance may be a complex phenomenon with many factors involved. Genetic changes may change other characteristics of the organisms.^{86,94} The organisms with acquired resistance sometimes appear to be a different species. Gram reaction may change from positive to negative; the changed organisms are resistant to other antibiotics; their forms may change to grotesque shapes or to filaments; they may lose their ability to ferment simple carbohydrates; or they may gain the ability to synthesize more aminoacids.¹¹⁶ Enzymes may be made to destroy the antibiotic. *E. coli* acquires greater ability to reduce chloramphenicol when it becomes resistant to that drug.¹⁸² *Bacillus cereus*, and penicillin-resistant strains of staphylococcus can be stimulated to produce more penicillinase (this enzyme catalyzes the hydrolysis of penicillin to the relatively inert penicilloic acid), by adding minute quantities of penicillin to the medium at 0°C prior to incubation at 35°C.^{87,153} Penicillin-sensitive organisms show no increase in penicillinase activity under similar conditions. Other organisms destroy penicillin by means other than penicillinase.¹⁶⁰ Organisms which have acquired resistance to one type of penicillin become resistant to other types.⁹² This resistance may be temporary. The growth rate, depressed as resistance is acquired, returns to normal as resistance leaves the strain of organism.⁹³ Nermut¹⁵⁴ suggests one way organisms become resistant is by losing the "cell envelope"; when they reform as a vegetative organism the sensitivity returns.

The gradual increase in resistance of organisms on a species-wide basis is of clinical importance for streptomycin and penicillin, while the data for other antibiotics is more encouraging. In a five-year study on bacterial acquired resistance to the three tetracyclines and chloramphenicol, Culbertson¹⁵⁷ found from examination of 2000 to 5000 strains each of *Staphylococcus aureus* (non-hemolytic), *Streptococcus* (non-hemolytic), *Streptococcus* (beta hemolytic), *Escherichia coli* and *Bacillus proteus* that they varied little from their original susceptibility, except for one or two quarters of one year (usually soon after the test period began). *Pseudomonas aeruginosa* became less susceptible to oxytetracycline and chloramphenicol for a period of two years. Huey and Edwards²⁰⁶ found about 5 per cent of cultures of *Salmonella typhimurium* recently taken from man were tetracycline-resistant, while none of those obtained prior to 1948 had shown resistance. Fungi do not develop resistance to nystatin.¹⁴⁴

Hussar and Holley¹⁶¹ have reviewed the question of acquired cross-resistance. Acquired resistance to one antibiotic may invoke no change

in the sensitivity of organisms to other antibiotics. Thus, no change is seen in the sensitivity to tetracyclines, streptomycin or bacitracin in organisms which acquired resistance to polymyxin; no cross resistance is usually found between streptomycin and penicillin, or streptomycin and the tetracyclines; and no cross-resistance is seen between the tetracyclines and chloramphenicol. On the other hand, resistance to other antibiotics may have simultaneously been acquired. This action, cross resistance, is expected and found most frequently when the second antibiotic belongs to the same chemical family; this is seen in the polypeptides (bacitracin does not participate here¹⁷³), the streptomycins and neomycin, the tetracyclines, and the macrolide (carbomycin and erythromycin) antibiotics.

Sometimes polypeptides and the streptomycins participate in cross resistance.¹⁸⁸ Cross resistance between tetracyclines and chloramphenicol is seen in gram-positive, but not in gram-negative organisms.

On the other hand, the acquisition of resistance to one antibiotic may make organisms more sensitive to another. Such is the relationship between streptomycin and the broad-spectrum antibiotics (the tetracyclines and chloramphenicol); resistance to one gives increased sensitivity to the other—a doublet in sensitization. Streptomycin as well as the broad spectrum antibiotics have been found to increase the sensitivity of organisms to penicillin, possibly through the suppression of penicillinase activity.

Multiple chemotherapy can lead to cross resistance simply by the process wherein an organism which has previously been made resistant to one drug is exposed to another (in the presence of the first). The resistance factors may be linked genetically; or the strains successful in adapting to the presence of one drug may have a greater potential to adapt to a second drug. At any rate, doubly-resistant strains arise more frequently than can be expected from single mutation rates.¹⁸³

d. Effects of Antibiotics upon Dependent Organisms

One sometimes gains the impression that given enough time some microorganisms use almost any available substance for some of their metabolic reactions. At a mutation rate of ten per billion, a liter of rich media might well contain 5,000 or 50,000 mutants; if one or more of them could make effective use of some unusual compound, such as an antibiotic, which the others could not use, the advantage would appear in progeny endowed with the same ability. It is known¹³⁴ that several loci of the chloramphenicol molecule are susceptible to enzymatic change to form a less active compound—the reduction of the nitro-group to arylamine

is accomplished by chloramphenicol-sensitive organisms. It is not long step for one or two of 50,000 mutants, from progeny which already detoxify a compound, to learn to use it as a good source for a reaction to produce a useful compound. Once the organism is producing the compound, enzyme systems previously used to produce it or a metabolically homologous compound from another source, may decrease, or disappear completely (mutation again). The new source material, the antibiotic, thus becomes a nutrient essential to the organism. The life of the organisms is then dependent on a source of that antibiotic or a bacterial mutation. Whether or not this is a true view of the mechanisms involved, many organisms have been found to be dependent upon various antibiotics. Thus far cross-dependency has not been confirmed.

Streptomycin dependence was seen in microorganisms by Miller and Bonhoff¹¹⁰ who found organisms which can grow only when this drug is present. A carbomycin-dependent mutant of *Micrococcus pyogenes* was distinguished by pink pigmentation. It required 12 mcg carbomycin per ml. of medium for maximum growth. Reverse mutation to carbomycin-sensitive, or carbomycin-resistant cells was found to be 1×10^{-6} to 1×10^{-9} .¹⁵⁵ Szybalski¹⁸⁸ reports both a penicillin-dependent strain and a partially chloramphenicol-dependent strain of *Micrococcus pyogenes* var. *aureus*.

Antibiotic-dependent organisms are useful in the search for cultures producing that antibiotic.⁹⁰ Gocke *et al*²¹⁴ also report a chloramphenicol-dependent organism.

The subject of stimulation by antibiotics is discussed in Chapter 3.

3. ACTION OF ANTIBIOTICS ON MULTICELLULAR ORGANISMS

The action of antibiotics *in vivo* is often different from their effect *in vitro*. This fact suggests that antibiotic-host interrelationships may be quite complicated. The increased *in vivo* effectiveness of the tetracyclines, chloramphenicol, streptomycin or penicillin may be partly due to the fact that at even sub-bacteriostatic levels these drugs make some bacteria more susceptible to phagocytosis.¹²⁶

Stimulation of growth in animals is noted when antibiotics are fed at sub-bactericidal levels: this phenomenon is discussed in the chapter on nutrition. Both the phenomenon and the mechanism are distinct and separate from the beneficial effect of adding a drug in bactericidal quantities to the feed of infected animals: such is the expected result. Morehouse *et al*¹⁶³ presented an early experiment in this field: giving arsenic acid in water to chicks heavily infected with coccidiosis allowed faster growth and better survival than was seen in chicks fed no drug.

is drug had previously been shown to be effective in outbreaks of coccidiosis.

The level of antibiotics added to animal feeds, about the year 1953, increased from 2–10 mg/Kg feed, a sub-bactericidal level, to 50–3000 mg/kg feed. This “preventive therapy” procedure is evidently needed under present systems of management with thousands of broilers reared in confined quarters. A clear presentation of this situation is given by White-Stevens¹⁶⁴: antibiotics in the feed prevent or overcome the infection, and the sick chicks gain weight faster. The feeding of therapeutic amounts of antibiotics may not be entirely beneficial, at least it is not in humans.

Of the various side reactions of antibiotics in humans, the most general seems to be the suppression of normal flora which may allow or stimulate a drug-resistant organism to establish itself predominantly in a “superinfection”. While many different organisms may be involved (see review by Jawetz¹³⁵), the one that has received the most attention is the frequently observed superinfection with *Candida monilia*.¹⁵² Huppert and Cazin¹³⁶ found that oral administration of bacitracin, chlortetracycline or neomycin increased the total growth of *Candida in vitro*. In mice inoculated with *Candida albicans*, all antibiotics tested (chlortetracycline, chloramphenicol, oxytetracycline, dihydrostreptomycin, magnamycin, neomycin, erythromycin, penicillin or tetracycline) showed increased number of *Candida* in the intestinal microflora. Since the action *in vivo* occurred with many antibiotics which had no effect *in vitro*, the results suggest that more is involved than a direct stimulation by the drug. This change in the status quo of the intestinal organisms appears to increase the susceptibility of the host to organisms resistant to the compound: e.g. streptomycin fed to mice makes them more susceptible to *Salmonellae* infection.¹³⁸ Foley and Winter also found increased mortality of chick embryos inoculated with *Candida* following penicillin therapy.¹⁵⁶ A problem equally serious to the host may be decreased antibody production by the host in the presence of antibiotics.¹³⁷

All antibiotics (except carbomycin) which are readily absorbed from the gastrointestinal tract are excreted in the urine (see Table 3–3, Chapter 3). Most of the others are also excreted *via* the urine. The efficiency of the kidney in excreting these strange molecules is one of the problems of the physician in his attempt to maintain effective blood (and tissue) levels to combat infection.

A brief summary of the toxicity of different antibiotics relative to their microorganism-inhibiting ability is provided in Table 1–6, taken from the wealth of data in the “Hand book of Toxicology,”⁸ which should be consulted for specific toxic reactions of each of the antibiotics. An

TABLE 1-6
ANTIBIOTIC TOXICITY

Antibiotic Organisms Route	Tetracyclines			Macrolides		Polyenes			Polypeptides				Miscellaneous				
	Tetracycline	Oxytetracycline	Chlortetracycline	Erythromycin	Carbomycin	Fungigallin	Nystatin	Rimocidin	Bactracin	Tyrothricin	Polymyxins, A,B,E.	Neomycin	Penicillin	Chloramphenicol	Novobiocin	Streptomycin	Cyclotexinide
LD 50 ¹	166	178	134		550		18	20	360	3.7	8	34	5900	245	407	200	360
Mice, I.V.	260		200						420	33	27	125		1320	281	9000	
Mice, I.P.	2600	7100	1500	2000	3500	2000	20,000		3700	1000		2900		2640	1000		
Mice, oral																	
<i>Inhibitory</i> ²																	
Microorganisms																	
Tissue culture, skin																	
Tissue culture, spleen																	
Relative toxicity (mice/microbe)	3300	89,000	67,000		55,000		14	4	180,000	600	400	170	59,000	4100	20,400	1000	1800

¹ Ave. mg. drug/Kg body weight.

² mcg/ml or mg/l.

approximate figure for toxicity of these compounds is given in the last line of Table 1-6. This figure is the ratio of the quantity of the compound which killed half of the mice (LD 50) to the amount required to inhibit sensitive microorganisms. In general most antibiotics have very low relative toxicity to mice. The polyene and polypeptide antibiotics have high relative toxicity, excepting bacitracin which is quite low. Cycloheximide, streptomycin, and chloramphenicol are intermediate in their relative toxicity to mice. The work with tissue culture indicates that a greater difference may exist between levels toxic to microorganisms and levels toxic to mammalian cells *in vitro*. This has led to their usefulness in tissue culture techniques. Their selective toxicity for different mammalian tissue cells may be useful, e.g., parascorbic acid prevents the growth of fibroblasts, but not that of epithelial cells.¹⁰⁷ Hundreds of potent antibiotics have been found which are too toxic to be considered for use in animals. Their use in plants is covered in a later chapter.

(E) MODE OF ACTION OF ANTIBIOTICS

At the present state of our knowledge, the mode(s) of action of antibiotics cannot be stated except as a series of part truths. Not only is the mode somewhat different for the reaction of each antibiotic and each reacting organism, but the action of the antibiotic upon the host or the environment of the organism must be known, or at least standardized, before the action of any one antibiotic can be defined, or compared with the action of another antibiotic. Since the myriad reactions have provided no standard rules, the remarks which follow are applicable only to individual experiments or to rough comparisons drawn from them. Recognition of such limitations gives some appreciation of the validity of the generalizations in this and later sections.

Interest in the mode of action varies in extent with the role that antibiotic is expected to play: the patient wants it to cure miraculously and completely; the physician wants to know which drug is bactericidal and which is bacteriostatic, or which is a broad spectrum antibiotic and which is not, or which kills bacillus *x* effectively without eliciting toxic reactions; the pharmacologist wants to know solubility, side reactions, interactions with other drugs, and effectiveness of related compounds. The microbiologist, on the other hand, looks more at the cell, asking a number of questions suggested by this point of view. Where does the cell break down, and why it is more susceptible to destruction in reproduction? Does the antibiotic produce resistant or dependent strains? Is it acting on the cell wall, the genetic apparatus or the size, shape and virulence of the cell? The physiologist considers the effect of the com-

pound on the respiratory system, on the growth cycle and on the use of energy from different sources. The biochemist wants to know what enzyme systems are affected, what compounds are bound to the antibiotic in the cell, and how, in turn, the cell affects the antibiotic. Not until the chemical equation for the reaction of antibiotic can be written, can the mode of action be said really to be known. Even then the facts about a very important point may be lacking. For it is indeed difficult to separate reactions in the cytotoxic sequence from secondary reactions which occur concomitantly or subsequently. Of the several reactions which might comprise a cytotoxic sequence, the primary interest centers around the initiating reaction.

In a general sense, all antibiotics act in the same way. They are either adsorbed onto or absorbed into the cell, and they all inhibit the effective growth of the culture in relatively low concentrations. They all exhibit some selectivity or differential toxicity to a variety of cells. In all, there is a continued integrity of many metabolic pathways until the whole cell is moribund or disintegrated—in other words there is a selective action of the antibiotic whereby it attacks some metabolic functions of the cell prior to others, or instead of others.

The mode of action of each antibiotic is different not only from that of other antibiotics but often from its own behavior under different conditions of use. However, while each has its individual mode of action, the number of different modes of action are limited, at least in the antibiotics which have been studied extensively.

In general, it is suspected that an antibiotic can act directly or indirectly in many possible ways: (1) on the cell wall to change its permeability or to cause lysis by increasing osmotic fragility; (2) on one or more enzymatic processes affecting the metabolism in absorption, in respiration, in energy utilization, in synthesis of genetic, structural or metabolic components, in detoxication reactions, or in maintaining the balance between enzymes, metabolites and products. Still other ways in which an antibiotic might act are: (3) on growth or accumulation of more "living substance"; (4) on the reproductive processes or chromosomal integrity; (5) on excretion processes; (6) on sensitivity mechanisms, increasing or decreasing them; (7) on information exchange processes; (8) on control of homeostasis in any of the other processes considered; and possibly (9) on integrity of defense systems.

We prefer to consider that the antibiotic acts without change in its essential chemical structure, which may, however, undergo hydration, esterification, ionization or absorption into a relatively specialized part of a colloidal system such as adsorption onto a protein, or absorption into a lipoidal layer.

The antibiotics which are classified as surfactants reduce surface tension and cause cytolysis, but there is no accepted explanation of their action. Other antibiotics are said to act as antimetabolites, i.e., compounds which prevent the normal functioning of an essential metabolite, usually by combining with the enzyme concerned in its disposition.

1. ANTIBIOTICS WHICH ACT AS SURFACTANTS

Many polar, lipid soluble compounds of widely differing structure reduce the surface tension of a solution by concentrating in surface layers or at interfacial surfaces or membranes, or by being adsorbed on certain parts of membranes or cell walls. Cytolysis may occur even when the amount of a surfactant is insufficient to give a monomolecular layer over the whole cell.¹⁶⁸ The surface energy of the cell membrane, or the interfacial tension of the system is greatly reduced by the presence of a surfactant. Exactly how this membrane is ruptured is apparently not well understood, and has not been extensively investigated. Some workers refer to the lipid material of the cell wall, others to the protein (and lipoprotein), and others point out that the cation is the essential element in cytolysis.¹⁶⁹ Presumably antibiotics which have surface tension reducing properties adhere selectively to cell walls, membranes, or interfaces, and reduce the cohesive force between the molecules which form that surface. The resultant reduction in surface energy increases cell permeability and the tendency of the membrane-forming molecules to disperse into the surrounding medium, to the extent that the integrity of the membrane may be lost. For lack of a membrane the cell is destroyed.

Surface-active bactericides have long been known, but are little understood. The statement that the mode of action of an antibiotic is by means of reduction in surface tension leaves much to be desired. A deeper understanding of the problem may be gleaned from the review by Newton.¹⁸⁹

The idea that surfactants kill cells by disorganization of their osmotic equilibrium mechanism should easily be refuted by treatment of bacteria in isotonic media. Such experimentation should clearly indicate that the change in osmotic pressure regulation is coincidental with the disintegration of the cell wall system. The observed change in cell permeability may be a direct effect of the action of the surfactant, or it may result from a secondary action. The surfactant may dissociate protein from prosthetic groups or nucleic acids. It may denature proteins, and thus directly affect enzyme systems, such as invertase, phosphatase, various dehydrogenases and cytochrome systems, which are associated with the cell wall.¹⁸⁹

The bactericidal activity of ionic surfactants is inhibited when compounds which combine with the ions are added (soaps, proteins, aminoacids, or phospholipids) before the detergent combines with the cell. In like manner anionic and cationic surfactants neutralize each other. A similar compound, phospholipid for example, present as a part of the cell wall structure, would provide a means of attachment of the surfactant to the cell wall.¹⁹⁴ If this were an ionic bond attachment, the effectiveness of the surfactant in reducing surface tension would be considerably decreased (if not reversed). One may suggest that the effective action of metal chelates in enhancing the activity of ionic surfactants is by keeping them oriented in their traditional working position, without being blocked by reaction with such cellular components.

Protoplasts from cells which have previously been treated with ionic surfactants are not osmotically fragile,¹⁹⁵ as are the usual protoplasts. Evidently the lipoprotein membrane remaining has been drastically altered by pretreatment with surfactant.

Anionic surfactants, such as soaps, bile salts, sodium lauryl sulfate (Dreft) and phenols, may be expected to orient on a cell wall with the acid portion of the molecule oriented into the extracellular fluid (since the acid group is hydrophilic), and with the hydrophobic lipoidal end oriented into the lipoprotein-complex part of the cell wall. Anionic surfactants are active against gram-positive organisms. Their effectiveness increases with acidity (which decreases their ionization). The most effective chain length for ionic surfactants is 12–16 carbon atoms. The mucocomplex of gram-positive bacterial cell walls is resistant to the action of ionic detergents, while anionic surfactants lyse gram-negative bacterial cell walls.

Cationic surfactants would be oriented in the same manner as the anionic detergents: the hydrophilic end toward the extracellular fluid and the hydrophobic part in the lipoprotein-complex of the cell wall. This group includes the quaternary ammonium salts, the polypeptide antibiotics (which have more basic than acidic aminoacids) and other organic compounds which are positively charged at neutral pH. They become more effective as the pH increases. These compounds are active against both gram-negative and gram-positive bacteria, although the polymyxins are generally more effective against gram-negative organisms.

Non-ionic surfactants usually contain polyhydric alcohols (e.g., Tween 80) or polyoxyethylene ethers (e.g., the Tritons). They have little known bactericidal action. The relation between activity and length of carbon chain is important in the action of these compounds. Cornforth *et al.*¹⁹⁰ found that compounds with chainlengths of 10–20

ethylene oxide units were antituberculous, compounds with 25–30 units had no activity, and compounds with 45–90 ethylene oxide units were protuberculous. The morphology of colonies is also changed by polyoxyethylene ethers: *Mycobacterium tuberculosis* changes from an amorphous growth to a cord-forming colony.¹⁹³

2. ANTIBIOTICS WHICH ACT BY METABOLIC INHIBITION (ANTIMETABOLITES)

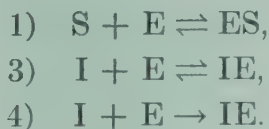
Although there is certainly too little data of quantitative nature to support the view that many antibiotics act as specific enzyme inhibitors, some have such properties, and others are suspected of being metabolic antagonists. Therefore a consideration of metabolic antagonism is essential for evaluation of the wealth of experimental data applicable to the mode of action of antibiotics.

In the usual metabolic reaction, a molecule (the substrate) is changed by a series of reactions into a different compound (product) which differs by one major component from the initial compound (addition of 2H, H₂O or $\frac{1}{2}$ O₂, removal of CO₂, etc.). Each such change is the result of a series of reactions which involve: (1) a combination of the substrate with a protein catalyst (the enzyme), (2) a change in the initial compound other than that implied by its attachment to the enzyme, and (3) a release of the product from the enzyme-substrate complex. (For the present the action of coenzymes or cofactors is not considered). The three reactions are shown by equations No. 1 and No. 2 where S is the substrate or the initial compound, E is the enzyme, ES is the enzyme-substrate complex, and P is the product:



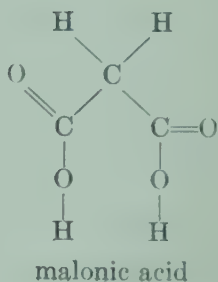
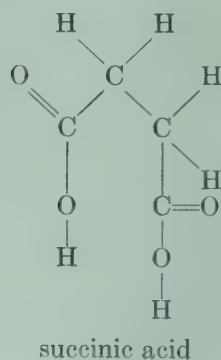
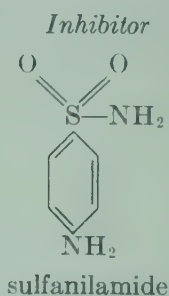
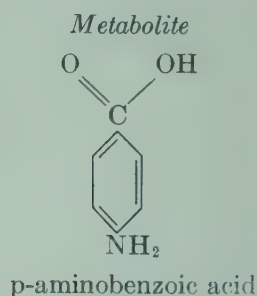
The arrows indicate that the enzyme catalyzes the reaction in either direction. The enzyme, as a catalyst, is essentially unchanged by the reaction, and is free to combine with another molecule of substrate. The enzyme does not initiate a reaction—it only increases the reaction rate. The specificity of an enzyme for the formation of the complex (reaction 1) is less than the specificity for completion of the reaction to form the product (reaction 2). Molecules which resemble the substrate in shape, size, chemical and electrical nature may react with the enzyme in reaction No. 1, then the overall reaction cannot be consummated to give the product by reaction No. 2. Therefore a strange molecule may inhibit the original reaction by lowering the effective enzyme concentration. The

inhibiting molecule (or antimetabolite) is designated as I in the following equations:

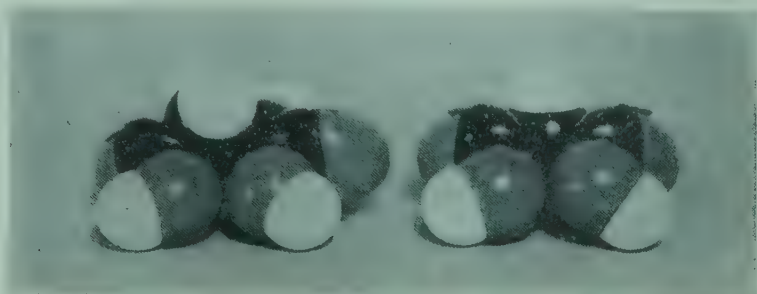


Two possibilities exist, as indicated in reactions No. 3 and No. 4. Reaction No. 3 is reversible, while reaction No. 4 is not. When both substrate and inhibitor are present, they compete for the same site on the enzyme (reactions No. 1 and No. 3). This is competitive inhibition. The velocity of the reaction depends upon the inhibition index and the actual concentration of inhibitor and substrate in solution. When enough inhibitor is added to decrease the reaction rate 50 per cent, the ratio of the quantity of inhibitor present to that of substrate is the inhibition index. This ratio is constant over a wide range of substrate concentrations. Beyond this range the relationship may change from competitive inhibition to non-competitive inhibition.

Classic examples of competitive inhibition are the inhibition of the utilization of p-amino benzoic acid by sulfanilamide, or the blockage of the succinic acid dehydrogenase reaction by malonic acid. As the formulas for these compounds indicate, the space occupied, general configuration, overall composition, and placement of similar reactive groups of the inhibitor is very similar to those of the actual metabolite. It is easy to see how the enzyme reacts with one as well as the other.



A photograph of models of either pair illustrates the point better than do the structural formulas, the fact being that there is so little difference between the pairs that the enzyme does not differentiate one from the other, and attaches to either compound. (See Figure 1-1). The enzyme-inhibitor complex cannot effect the next step in the reaction series. The success of the sulfa-drugs as competitive antimetabolites has greatly influenced thinking and research in antibiotics. Although several antibiotics are found to be competitive inhibitors of certain metabolites (see the discussion of specific action of antibiotics later in this chapter), it has not been shown that this is their mode of action as a selective inhibitor.



Succinic Acid

Malonic Acid

FIGURE 1-1. Metabolite and Competitive Inhibitor.

In non-competitive inhibition, reaction No. 4, breaking of the enzyme-inhibitor complex, occurs slowly, if at all. Therefore in the presence of this type of inhibitor, the addition of more substrate cannot effect increased formation of ES. Minute quantities of heavy metals such as Ag^+ , Cu^{++} , Hg^{++} , Pb^{++} or arsenicals may act in this capacity against enzymes which depend upon sulfhydryl groups for their activity.

Haenel²¹² has proposed a simple test to distinguish between competitive and non-competitive inhibition, as illustrated in Figure 1-2. An agar plate is flooded with uniform inoculum of an organism which requires metabolite M. Metabolite M is placed on the agar in two porous cups, and the inhibitors are placed on the agar at some distance from the metabolite. Material diffuses from the four cups to give a concentration gradient of the material in the agar. The organism grows well when it receives M. The non-competitive inhibitor (N) blocks all growth wherever it reaches, to give a complete circle of inhibition. The competitive inhibitor has a circle of no growth around it, which is interrupted as the metabolite concentration changes—this effect produces a straight line of growth demarcation between the two.

Much has been written regarding the effect of antibiotics in disrupting or decreasing thiol groups (SH groups) of the bacterial cell. Many

enzymes depend upon integrity of the thiol groups for intramolecular function or for interaction with other molecules. These include urease, papain, myosin, β -amylase and succinoxidase.¹⁶⁵ This reaction may be generalized as $2\text{RSH} \rightleftharpoons \text{RSSR} + 2\text{H}$. This reaction also occurs intramolecularly in a single molecular species, as seen in cystine-cysteine, and thioctic acid (when it carries hydrogen). Changes from reduced

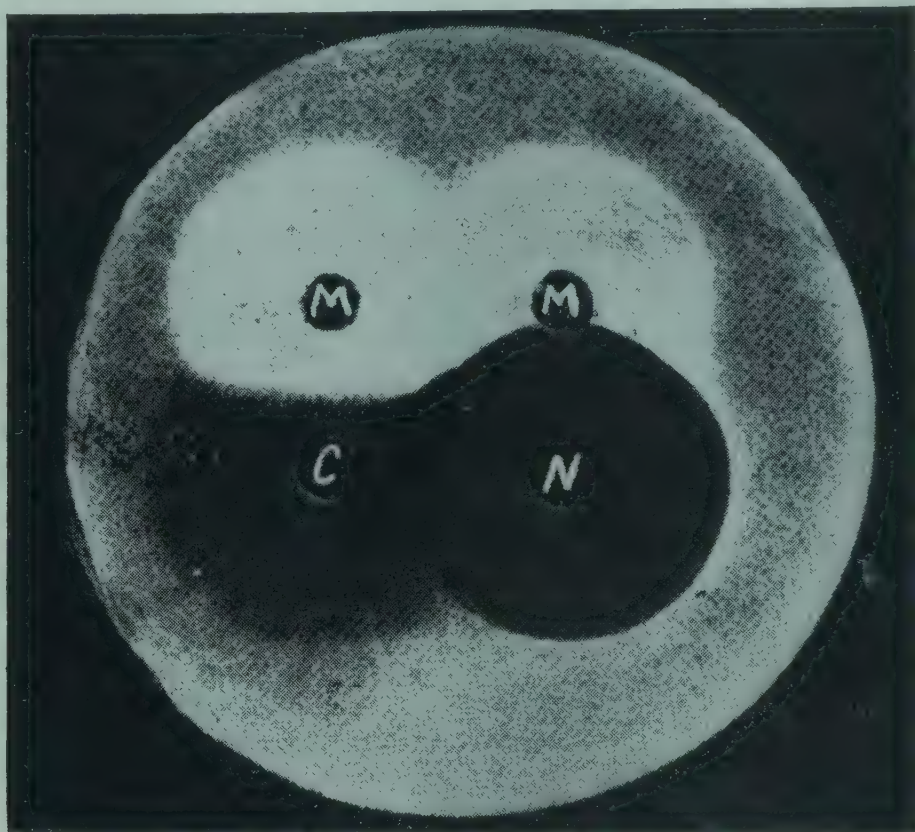


FIGURE 1-2. COMPETITIVE AND NON-COMPETITIVE INHIBITION. Plate inoculated with *Phycomyces* sp. in nutrient agar without thiamin. The essential metabolite (thiamin) is added at M; the competitive inhibitor (butylpyrithiamin) added at C, and the non-competitive inhibitor (HgCl_2) added at N. Note the relatively straight line formed where the metabolite counteracts the competitive inhibitor, in contrast to the lack of effect of the metabolite upon the diffusion circle occupied by the non-competitive inhibitor.

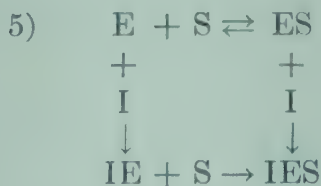
to oxidized glutathione in biofluids (GSH/GSSG) which have been followed in medicine for half a century, and the two chains of insulin held together by disulfide (S-S) linkages illustrate the importance of this reaction in the molecular plan of life. Intermolecular reactions are more varied but they often may be represented in simplified forms as:



If R' be an acid, this reaction would represent the formation of a compound such as acyl-Co A (where RSH represents coenzyme A).

Thus, proof of antibiotic interference with the sulfhydryl system would have accepted importance. Many enzymes could not maintain their working integrity, certain coenzymes could not react with their substrates, and sulfhydryl hydrogen carriers could not function. Pratt's theory of penicillin action (given more fully in the next section of this chapter under specific action), assembles convincing evidence for this hypothesis. He suggests that some antibiotics may act by decreasing the availability of essential thiol groups. This evidence is based on the fact that the inhibitory action of certain antibiotics is overcome by strong reducing agents, such as ascorbic acid, cysteine, or glutathione. The effect of dehydroascorbic acid in bacteriostasis, while the enediol form has no bacteriostatic activity,²¹⁰ would support this view. Krampitz and Werkman¹⁰⁶ observe that this action alone would not account for the observed specificity of the antibiotics. Thiol-binding agents are general cytotoxic agents with little or no selectivity exhibited toward the genus or species of the cell eliminated. The fact that different enzymes do not have the same sensitivity to any one agent, plus selectivity in absorption of that agent, could produce specificity of action. A similar argument must be presented if we are to accept the important suggestion of Kraskin and Stern¹⁵¹ that oxytetracycline acts as a competitive inhibitor of DPN.

Uncompetitive inhibition is the condition wherein the inhibitor attaches to the protein at a site different from that binding the substrate. It is similar to noncompetitive inhibition in that it is relatively non-reversible, and in that the addition of excess substrate cannot drive the reaction to completion. The complex IES may be formed (reaction No. 5) in either of two ways:



The end result is that the inhibitor and substrate both combine with the enzyme, at different sites, and that the presence of the inhibitor blocks its further reaction to produce the product.

The above examples often show great specificity as far as the enzyme involved is concerned. Non-specific inhibitors exist which ruin enzymes simply because they are protein denaturants. Non-specific inhibitors

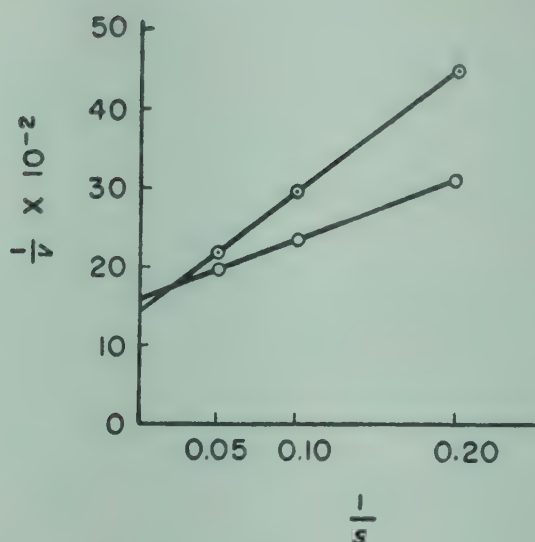


FIGURE 1-3. Lineweaver-Burk plot of terramycin inhibition of methylene blue reduction in the presence of substrate, diphosphopyridine nucleotide (DPN).

○ -- ○ --, 200 mcg/ml terramycin.

○ -- ○ --, 100 mcg/ml terramycin.

include phosphotungstic acid, strong mineral acids, trichloroacetic acid, heat, oxidation and even proteolytic enzymes.

Mathematical treatment^{165,166} of the data is usually needed to distinguish between competitive and non-competitive inhibition. Kraskin and Stern have shown the competitive nature of the inhibition of the coenzyme DPN by oxytetracycline using the Lineweaver-Burk plot as given in Figure 1-3.¹⁶¹

If the antibiotic is assumed to block one essential metabolic reaction, its action should be represented by one of the mechanisms shown in Figure 1-4, which gives a simplified and essential sequence of reactions. Each letter represents one metabolite, and each number represents the reaction, or series of reactions, leading from one compound to the other. Antibiotics which affect the same reaction may act synergistically. A compound (antibiotic) which inhibits or blocks a reaction preceding

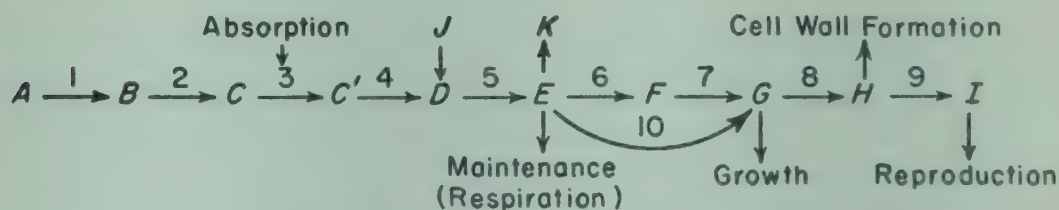


FIGURE 1-4.

another reaction in the same biological sequence may be antagonistic to the antibiotic which affects the latter reaction, because the organism never obtains enough of the materials entering into latter reaction for the antibiotic to act successfully. The reactions are not necessarily linked chemically in one long chain, but represent possible essential steps in the perpetuation of the species. In every case one or more of the compounds must be available in the medium, and interference with the absorption (reaction 3) of that compound may be the initial cytotoxic reaction. Reactions 1 and 2 represent the preparation of compound C for absorption—extracellular enzymes splitting it from a biological polymer (a protein, a polysaccharide or a nucleotide), or phosphorylating it at the cell wall surface. Antibiotics may interfere with reaction 2 simply by combining with the essential metabolite to prevent its absorption. Such may be the action of polymyxin with trace elements such as Mn^{++} . An antibiotic which interferes with the absorption of a compound (e.g., by reaction 3 which brings metabolite C' into the cell) may stop the sequence of reactions in a susceptible organism which thus cannot make compound D. Reaction 3 may also be blocked in a resistant organism which can produce compound D from another system of reactions as it was needed. An example of this type may be the adsorption of glutamate which is blocked by penicillin, an action which affects the synthesis by glutamate of organisms only slightly, if at all.

As the reaction $J \rightarrow D$ comes into play, the enzymes for this series of reactions may be accumulated in greater quantity (activity), and the enzymes for the reactions $C \rightarrow D$ may decrease. This would represent adaptation by the cell to the presence of the antibiotic, wherein the cell acquires greater resistance to the antibiotic. It is also possible that addition of D to the medium could affectively bypass the block on reaction No. 3. (See the discussion later of the effect of adding phenylalanine to chloramphenicol-inhibited cells).

Each reaction usually has products other than the metabolite needed for the essential sequence. These byproducts, represented by K, must be disposed of, or else their accumulation may slow an essential reaction, or produce an unhealthy intracellular environment. While the organic acids are obvious examples of this process, too much of any single compound may be harmful. An antibiotic may work by preventing disposal of such waste.

If one antibiotic blocks reaction 10 at the same time that another antibiotic blocks reaction 7, the cell is inhibited more effectively than if either antibiotic were present alone, for the double action means that the cell can not use either pathway. This process represents antibiotic synergism. The effect of antagonistic antibiotics is shown diagram-

matically in Figure 1-5. Antibiotic I slows or stops the sequence of reactions from going to reaction 9 where antibiotic II can act. Antibiotic I thus prevents effective action by antibiotic II. Eagle¹⁵⁹ suggests a similar explanation for the decreased effectiveness of excess penicillin: a high concentration may block a reaction which occurs earlier in the chain of metabolic events, and is less essential to the life of the organism than the later reaction, which would be effectively blocked by lower concentrations of penicillin.

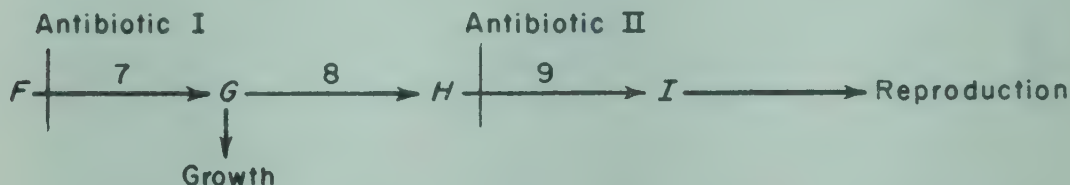


FIGURE 1-5.

If antibiotics act as indicated, one might expect to find specific anti-metabolites which would enhance (or antagonize) the action of antibiotics. While this appears to be a fruitful research area, it must be acknowledged that some antibiotics act in a less specific manner.

3. ACTION OF SPECIFIC ANTIBIOTICS

a. Mode of Penicillin Action

The extensive work with penicillin is both an inspiration, and a model for further work on other antibiotics.

Pratt and Dufrenoy⁹¹ review the action of penicillin upon bacterial cell morphology. Bactericidal levels of penicillin cause cell distortion, concatenation, swelling and lysis. The resultant release of intracellular constituents effectively supplies nutrients which may stimulate the growth and metabolism of neighboring cells, and hence contribute materially to their increased sensitivity to penicillin and death (since microorganisms in the logarithmic phase of growth are most sensitive to penicillin). The concatenation in *Escherichia coli* and *Bacillus subtilis* is manifested as elongated mycelium-like structures, in *Staphylococcus aureus* as streptococcal-like chains, and in streptococci as longer chains.

The cells may swell and occasionally divide, but penicillin-treated cells usually fail to produce daughter cells. Low levels of penicillin produce filamentous forms which suggest¹⁴⁷ that the division septum is particularly sensitive. Work of Lederberg,¹¹⁸ and electron photographs, taken by Pease,¹⁴⁸ of sensitive organisms grown in the presence of penicillin show that L-forms have a lesion in the cell wall, the insides are ejected

(much as a child blows large bubble-gum spheres) as a protoplast which can be subcultured in a special penicillin medium. The mucocomplex of the cell wall is reduced, while about one-half of the antigenicity (lipoprotein?) remains. The cell is made osmotically fragile, since the protoplasts formed will lyse in ordinary broth. (These little cells "have heavy buttons on their overcoats but their BVDs are equipped with zippers"). When penicillin is removed from the environment, the bacillary form is regained and can be subcultured.

From a review of the work on radiopenicillin, the site of action of penicillin is also suggested as being within the cell wall, where a small quantity of penicillin is irreversibly bound. Cooper⁸² cites evidence that a penicillin-binding component (PBC)⁸³ is present in penicillin-sensitive strains, and is not present in resistant organisms (except at low levels). Sensitive organisms bind penicillin to raise the cellular concentration above that of the medium at low levels of penicillin, here the rate of binding corresponds to the growth rate.

The site of binding is specific for penicillin. Since many penicillin derivatives and surface-active agents neither prevent penicillin uptake nor remove bound penicillin; the amount of bound penicillin increases with the growth of the organism. The rate of binding on the cell wall is very rapid (2 minutes) to saturation (a small consistent quantity). Since only a small fraction of penicillin is found as compared to the total capacity of the cell to bind penicillin, it seems apparent that penicillin does not usually penetrate very far through the cell wall, if at all.⁸⁴ From work in which formalin fixation precedes rupture of the wall, Cooper⁸² suggests that the penicillin-binding component is associated with the lypoprotein of the cell wall. The fact that material from penicillin-resistant organisms also binds penicillin when the cell wall is broken suggests that there is an essential cell wall phenomenon in the mode of action of penicillin.

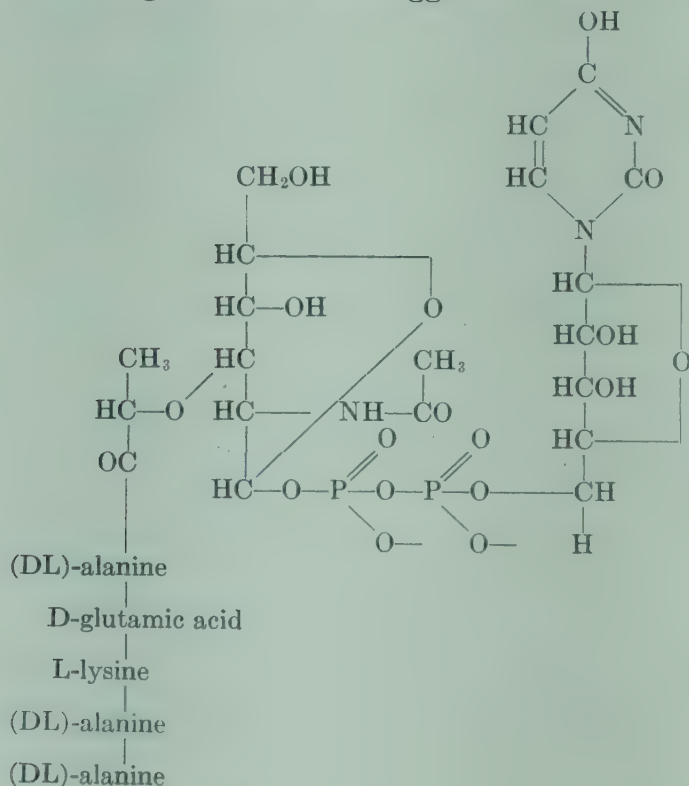
Maas and Johnson⁸⁴ find that cells may bind about 750 molecules of penicillin per cell with no effect on growth. When 1500 to 1700 molecules per cell are bound, growth inhibition sets in, and when 1500–2400 molecules per cell are bound a bactericidal action occurs. In organisms which produce penicillinase, the rate of production is correlated closely with the amount of penicillin irreversibly bound. Both reach a maximum at about 10 mcg/ml of penicillin. Cooper suggests a time-table for the action of penicillin, which is helpful in correlating different effects of penicillin in the medium.

The significant work of Park, first with M. J. Johnson and later with J. L. Strominger, gives insight into a basic lesion of the penicillin-treated cell.¹¹⁷ Several uridine nucleotides were found to accumulate in the

ACTION OF PENICILLIN

Time, min. (and stage)	Result
0-2 (Reaction)	Penicillin (up to 0.1 mcg/ml) binds irreversibly ¹⁴⁶ to all surface penicillin-binding component (PBC) present. Bacterial reproduction ceases.
2-30 (Bacteriostasis)	No deaths or no lag in growth occurs if penicillin is removed. Amount of penicillin bound is doubled, with a concomitant loss of internal penicillin-binding component. Rate of growth is slower after this experience. Helvolic acid prevents permanent damage during this period. Glutamic acid absorption is decreased. ⁹⁵
30-60 (Bactericidal)	Viable count decreases exponentially during this period. Binding of penicillin continues at a decreasing rate. Cells abruptly cease to accumulate Na^+ , Mg^{++} , K^+ , H_2PO_4^- , glutamate, or gross dry weight. Uptake of Co^{++} and Fe^{++} continues. Removal of penicillin prevents more cells dying, but the lag in growth resumption increases with the duration of penicillin contact. Synthesis of cell protein and cell wall material stops. Synthesis of peptides, phospholipid and nucleic acid continues.
60-75 (Degeneration)	Nucleic acid ¹⁰⁸ and peptide synthesis slow down.
75 (Death)	Cells lose solutes and swell, while phosphate incorporation into large molecules decreases. Cell may burst.

penicillin-inhibited cell. The main component is a uridine nucleotide for which the following structure was suggested:



This nucleotide carries a complex very similar in its composition to that of the cell wall itself. The unique sugar moiety has been found only in bacterial cell walls and in this accumulated material. A comparison of the components of each system is given in tabular form.

Compound	Nucleotide	Cell Wall
Glutamic acid	1	1*
% D-glutamic acid	100	92
Alanine	3.0	3.35
% D-alanine	52	45
Lysine	1.0	1.06
3-0-carboxyethyl hexosamine	1.0	.93

* Quantities of components are expressed in terms of the molar quantity of glutamic acid, which is arbitrarily assigned a value of unity.

Parks and Strominger conclude that this complex uridine nucleotide is the biosynthetic precursor of the bacterial cell wall. Since high-energy uridine pyrophosphate glycosyl compounds can transfer the sugar unit to the other compounds, these investigators regard this compound as an activated intermediate in the biosynthetic transglycosidation occurring in the formation of the cell wall. They suggest that penicillin specifically inhibits the enzyme (transglycosidase), which utilizes this compound in building the cell wall. This would explain both the decreased glutamate adsorption and the selective toxicity of penicillin for those cells which build such a cell wall. Animal cells are immune to the action of penicillin because they do not make a comparable cell wall. Although this theory does not explain the status of resistant microorganisms, such proposed mechanisms show that one of the most important results of antibiotic research is the increased knowledge of cell structure and metabolism.

Pratt and Dufrenoy⁹¹ suggests that penicillin promotes the oxidation of sulfhydryl groups faster than they can be replaced, and that decrease in the reducing capacity of cells enhances penicillin activity. The ratio of RSH to RSSR compounds appears to be important to the efficient action of penicillin, which may be regarded as being activated under oxidizing conditions and inactivated under reduction, as when conditions cause the reaction $2\text{RSH} \rightleftharpoons \text{RSSR} + 2\text{H}$ to go to the left. Compounds which undergo this reaction are cysteine, glutathione, lipoic acid and proteins. The action of penicillin is enhanced by cobalt⁹⁷ and methionine⁹⁶ (which can overcome the depressing action of glutamate), and also by a high oxygen potential (positive eH) within the cell at the time for dividing (as shown by vital stains).⁹¹ The effectiveness of penicillin is a direct function of O_2 tension.⁹⁹ The action of penicillin is depressed by hydrogen pressure⁹⁹ (a reducing medium), glutamate⁹⁶ and cysteine,⁹⁸

both of which are constituents of glutathione, while cysteine itself would contribute to make the general thiol pool larger.

Knight cites the reverse case which fits the Pratt hypothesis. Spores of an obligate anaerobe (*Clostridium tetani*) placed in a medium with no free O₂ or SH groups (RS-SR compounds were present), did nothing. When cysteine or glutathione were added, they germinated and divided so fast that protoplasm was not produced fast enough to keep up with reproduction so the rods looked more like coins, that is, the cells maintained their normal width but not their length.¹⁰⁵ Under the influence of penicillin *S. aureus* loses its gram positive character.⁹⁵

b. Mode of Action of the Polypeptide Antibiotics (polymyxins A, B, C, D, and E, subtilin, tyrocidin, circulin, bacitracin, gramicidins)

Surface-active polypeptides, such as the polymyxins, apparently have the ability to disorganize the cell wall and kill a microorganism within a few minutes. The amounts of these bactericidal surfactants needed varies with the size of the inoculum,⁷³ which suggests that the compounds are absorbed by the bacteria. The effects of various cationic detergents are compared with those of polymyxin by Newton,⁷⁴ who found that water-soluble constituents, such as purines and pyrimidines, are released into the media after the addition of polymyxin to sensitive organisms. No material was released from resistant microorganisms. *A. aerogenes* is much more susceptible to polymyxin B when the medium is rich in aminoacids—particularly leucine and serine.¹⁷⁴ Further evidence of attachment upon the cell wall was furnished by the addition of a dye which forms a fluorescent compound with negatively-charged groups in protein. The dye showed fluorescence in the presence of washed bacterial cells only after polymyxin was added.⁷⁵ Since the addition of polymyxin caused an immediate reaction with the dye,⁷⁹ but only a slow release (to 5 hours) of intracellular constituents, it is evident that more than one part of the process is represented by these two observations. Newton suggests⁷⁴ that the antibiotic immediately affects cell permeability, which leads to the eventual disruption of the cell wall. However, the possibility that the dye is reacting with the polypeptide has evidently not been ruled out, although an excess amount of the dye did not increase the maximum intensity of the fluorescence. High concentrations of polymyxin inhibit autolysis of cells. Electron-micrographical work substantiates the idea that polymyxin alters the cell wall structure, with loss of cytoplasmic elements as shown by the appearance of "ghost cells", while high concentrations of polymyxin produce cells with ragged edges which have maintained their full quota of electron-stopping material.⁷⁶

While polymyxin has been shown to inhibit many enzyme systems, such as esterase, and hence, or also, the oxidation of acetate, pyruvate, oxalacetate, and 2-ketogluconate, at bactericidal levels these systems are not affected.⁷⁴ Even at a level of polymyxin three times bactericidal (3B), *Pseudomonas aeruginosa* oxidized glucose to 2-ketogluconic acid. This agrees with the finding of Sikes⁷⁷ that surface active agents affect viability at concentrations lower than those which inhibit catabolism.

Greater quantities (about 4-fold difference) of polymyxin are absorbed by polymyxin-sensitive than by polymyxin-resistant bacteria; this is also true of suspensions of their cell walls.⁷⁸ Few has noted⁸¹ that polymyxin-resistant organisms appear to have a single-layered cell wall, while polymyxin-sensitive organisms have an inner layer. Evidently the protoplasmic membrane also absorbs polymyxin, since Newton found a fluorescent derivative of polymyxin in the protoplast membrane of lysozyme treated cells of *Bacillus megaterium*. *P. aeruginosa* had the antibiotic derivative distributed equally between the inner and the outer coats. Burdon⁸⁰ gives evidence that this layer contains predominately lipid, and Few⁸¹ has shown that lipid, and cephalin, extracted from *Pseudomonas denitrificans* complexes strongly with polymyxin. Analytical data of the cell walls of bacteria suggest that polymyxin-sensitive bacteria have more phospholipid than polymyxin-resistant bacteria.⁷⁴ Presumably these phospholipid compounds are not lecithins, since choline was not found. Unfortunately, there are no data to indicate that the place where the antibiotic is found in the dead cell is in the essential site of action. Acquired resistance is rarely found. Polymyxin and circulin produce reciprocal resistance.¹⁷³

Cationic detergents increase the antibacterial action of these toxic peptides,¹²³ while it is decreased by anionic detergents¹²⁵ or magnesium.¹²⁴ Evidence also indicates that tyrocidin^{168,169} and subtilin¹⁷⁶ act as germicidal surfactants.

The cyclic peptides may be more effective in living systems than straight chain peptides because they are resistant to aminopeptidases, carboxypeptidases and possibly other proteolytic enzymes. Thus pentapeptides having the same aminoacid sequence as gramicidin S, a cyclic decapeptide antibiotic, were found to have no activity.¹⁹¹ They also have a more rigid configuration in the cyclic form. When Erlanger *et al.*¹⁹² synthesized a straight-chain decapeptide with its aminoacid sequence identical to that of gramicidin S, it was active until the δ -amino group of L-ornithine was bound. Similar results⁷⁹ with polymyxin peptides and tyrocidin indicate the presence of the free amino groups (those not engaged in the amide linkage making the antibiotic) is essential for the cytotoxic reaction. Gramicidin inhibits uptake of phosphorus.¹⁷⁵

Marked concatenation occurs in organisms under the influence of bacitracin evidently to a greater extent than under the influence of penicillin, sulfonamides or norvaline.⁹¹ Bacitracin is similar to penicillin not only in that it induces similar morphological changes, but also because it is bacteriolytic, forming protoplasts under proper conditions (hyper-tonic solution).¹⁸¹ A more striking similarity is the accumulation¹¹⁵ of uridine nucleotides in cells damaged by bacitracin (or cephalosparin N or C) similar to that seen in penicillin-treated cells. The present simplified view would suggest that these compounds act at a common site in the maintenance of the cell wall of gram positive organisms.

c. Mode of Action of Chloramphenicol

The reactions of chloramphenicol on biological systems indicate that there is little effect on absorption of glutamate by the cell wall, although inhibition (complete at high levels) of the incorporation of glutamate into protein was found. Protein synthesis stopped, while no change was seen in glucose fermentation by *Staphylococcus aureus*. Low levels of chloramphenicol drastically reduce protein synthesis in sensitive bacteria, while higher levels affect respiration, glutamic acid and phenylalanine accumulation, and nucleic acid synthesis.²⁰⁹ Chloramphenicol also decreases the assimilation of ammonia by bacteria.¹⁰² Glucose uptake and metabolism are decreased in *E. coli* on treatment with chloramphenicol.¹⁹⁶

Hahn, *et al*¹⁵⁰ suggest that the effect of chloramphenicol on protein synthesis is not because the high-energy compound, adenosine triphosphate (ATP), is not synthesized or utilized, since the drug failed to influence energy metabolism in systems of bacterial (1) bioluminescence, (2) phosphorylation, and (3) motility. Chloramphenicol was found to have no viricidal action against T-1 bacteriophage, nor did it prevent adsorption of the virus into the bacteria. It did stop the intracellular growth of the virus, presumably by its inhibition of protein synthesis.¹⁴⁹

The bacteriostatic action of chloramphenicol is less effective in activity-metabolizing bacteria—a rich medium decreases the effect of this drug. Eagle and Saz¹³² review the counteraction of low levels of chloramphenicol by aromatic amino acids, as well as the action of chloramphenicol in the inhibition of tyrosine and phenylalanine oxidation and deamination. Since anthranilic acid does not counteract chloramphenicol, while tryptophane, indole and tyrosine do, this drug may inhibit the synthesis of aromatic derivatives from anthranilic acid.^{133, 121} Hopps *et al* suggest that chloramphenicol does not act as a simple amino-acid antagonist, since they could not reverse its action by aspartic acid,

glycine, phenylalanine or tryptophane.¹⁴² Woolley¹⁶⁷ presents evidence showing that chloramphenicol inhibits phenylalanine metabolism on a non-competitive basis, while several compounds related structurally to chloramphenicol were competitive inhibitors when present in small amounts.

Many organisms can change the chloramphenicol molecule, sometimes even chloramphenicol-sensitive organisms can do so. Presumably some of the products may not only be less toxic to the organism, but they may also counteract the residual drug. A list of substituted phenols which counteract chloromycetin *in vitro* is presented in Table 1-7. The

TABLE 1-7
EFFECT OF SUBSTITUTED PHENOLS IN COUNTERACTING
CHLORAMPHENICOL¹⁶⁷

Compound	% normal growth
Chloramphenicol	50
o-nitrobenzaldehyde	60
3,4-dichlorobenzaldehyde	62
p-nitroaniline	63
p-nitrotoluene	64
o-nitrophenol	65
p-hydroxybenzaldehyde	65
p-dimethylaminobenzaldehyde	70
p-nitrobenzaldehyde	75
2,4-dichlorophenol	75
p-nitrochlorobenzene	76
2,4-dinitrophenol	76
m-nitrobenzaldehyde	80
2,6-dinitrophenol	81
2-amino-4-nitrophenol	90
tyrosine	98

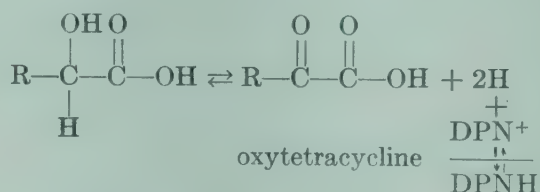
evidence suggests that other compounds which should approach tyrosine, tryptophane or phenylalanine in their effectiveness may be 2,4-diamino phenol, 3-hydroxy-kynurenine or 3,4-dihydroxy (1-hydroxy-2 methyl-aminoethyl) benzene, which is epinephrine. (The latter compound could lead to an interesting hypothesis if bacteria had nerves). Ciak and Hahn¹⁹⁸ suggest that the action of chloramphenicol and tetracyclines is additive.

d. Mode of Action of Tetracyclines

The fact that oxytetracycline, chlortetracycline, and tetracycline are so similar in their chemical structure and properties indicates they may be expected to have similar bacteriological and biological behavior. This expectation is borne out by the finding that development of resistance to one of the drugs increases resistance to the others,¹⁰⁴ and by the

observation that they do not usually give additive effects. The foregoing statement is not always true; thus the work of Snell *et al*²¹⁵ indicates that the tetracyclines may act differently. In general, the tetracyclines are bacteriostatic in low levels and bactericidal at tenfold higher concentrations. Tetracycline-dependent strains have not been reported. Glycine or cysteine can counteract some of the effects of the tetracyclines, which may be evidence that chelation is important in the action of the tetracyclines.¹⁹⁷ Some gram negative organisms produce material which counteracts chlortetracycline.¹⁹⁹

The specific reactions reported as involving the tetracyclines are summarized by Eagle and Saz.¹³² In bacterial cells inhibited by one of these drugs, respiration is decreased (particularly in the logarithmic phase of growth) while carbohydrate oxidation is diminished at both the glycolysis level and the tricarboxylic acid cycle level. Acetate oxidation is very sensitive to these inhibitors, and the utilization and deamination of phenylalanine, tyrosine and phenylalanine is decreased. Tetracyclines inhibit the action of urease, and decrease protein synthesis in low concentrations, and nucleic acid formation in higher concentrations. While folic acid and vitamin B₁₂ may prevent the latter action, tetracyclines and other antibiotics inhibit the synthesis of these vitamins by *E. coli*. D-glutamate accumulates in the broth of *E. coli* cultures treated with oxytetracycline.²¹⁵ Chlortetracycline and oxytetracycline inhibit phosphorylation, or even reverse the phosphorylation reaction (from respiration) at lower levels¹²² than those which affect respiration.¹⁰³ Chlortetracycline inhibits glutamate transport into cells.²⁰⁰ Kraskin and Stern¹⁵¹ showed that the oxidation of gluconate to 2-ketogluconate is inhibited by oxytetracycline, which acts specifically as a competitive inhibitor to the coenzyme, DPN (diphosphopyridine nucleotide). This work suggests that the reason for the decreased oxidation of other substrates is due to the general participation of DPN as a hydrogen carrier. The action as outlined below shows that tetracycline has no direct effect on the enzyme or on the compound being oxidized:



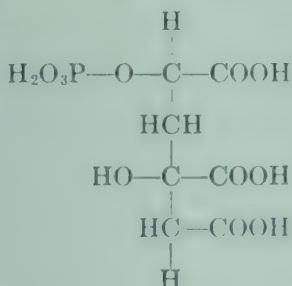
If this reaction is to be regarded as the cytotoxic reaction of tetracycline, one must explain its specificity to susceptible cells on the basis of decreased absorption into the cell, of increased quantity of DPN⁺ or of the inactivation of oxytetracycline by resistant cells. This work will not

be important unless it leads to results which may be obtained at bacteriostatic concentrations (about 100,000 times lower than those used thus far). Oxytetracycline selectively catalyzes the oxidation of ascorbic acid; if H_2O_2 is the end product, this reaction may be the cytotoxic reaction.²⁰⁸ The high affinity of oxytetracycline and chlortetracycline for metallic ions,²¹¹ and the effect of high concentrations of Mg^{++} or other ions in inhibiting the action of tetracyclines and the polypeptide antibiotics, suggests the thought that these compounds may act by competing with cations for adsorption sites on or in the cell, or by chelating with the ions.

e. Mode of Action of Streptomycin

Streptomycin may kill resting as well as growing organisms. However, *E. coli* is more susceptible to streptomycin when it is in the growth phase.¹³⁰ It inhibits respiration, but it also kills anaerobes. It inhibits the formation of chlorophyll in *Euglena gracilis* as well as in seedlings.¹³¹

One mode of action of streptomycin and related compounds appears to be interference with respiration,¹⁷⁹ which is particularly effective when the organism metabolizes exogenous substrate.¹¹⁹ One basic lesion appears to occur in the terminal oxidation cycle; thus pyruvate fails to condense with oxalacetate, and acetate accumulates. Umbreit¹²⁰ suggests that the organism normally would condense the 3-carbon atom pyruvate with the 4-carbon atom oxaloacetate to form the 7-carbon atom metabolite isolated by Rappaport and Wagner.²¹⁶ This compound (2-phospho-4-hydroxy-4-carboxy-adipic acid)



represents the beginning of an oxidation cycle which is stopped by the addition of streptomycin. Streptomycin inhibits the formation of this compound, and the sensitive organism cannot maintain its normal energy supply. Resistant organisms bypass this particular metabolic step,¹⁷⁹ and usually oxidize very little pyruvate. The selectivity of action of the drug may be primarily a matter of which cells absorb streptomycin, since the condensing reaction is stopped by streptomycin *in vitro* when animal enzymes are used. Such a mechanism would not explain its

toxic action on organisms which do not use a terminal oxidation cycle (e.g., the tricarboxylic acid cycle) for energy metabolism. Streptomycin also inhibits,¹²² the reactions of diamine, oxidase and inositol metabolism, and the synthesis of pantothenic acid. The drug complexes with nucleic acids and nucleoproteins, and it apparently penetrates slowly into mammalian cells.

Since resistance of bacteria to streptomycin can be obtained by adding casein hydrolysate or the proper combination of amino acids, streptomycin may inhibit the synthesis of these amino acids within the cell. When they are supplied in the medium sensitivity decreases.¹⁷⁴ B-vitamins may prevent the cytotoxic effect¹²⁹ or the growth inhibition⁶² of streptomycin. Its lethal effect is inversely proportional to the concentration of phosphate in aerobic media.²⁰²

The action of dihydrostreptomycin appears to be similar to that of streptomycin.

f. Mode of Action of Other Antibiotics

Nystatin inhibits endogenous respiration in fungi, and their utilization of glucose, whether aerobic or anaerobic.¹⁴⁴

Erythromycin and carbomycin act only during multiplication of bacteria.

Novobiocin acts differently against gram positive and gram negative bacteria.¹⁴³ Its activity against the latter, in which the drug produces filamentous forms, is counteracted by magnesium sulfate. With gram positive bacteria the antibiotic causes no filamentation and magnesium sulfate has no effect. Ribonucleic acid (RNA) synthesis is not reduced.

Cycloheximide inhibits growth and fermentation in yeast cells, while it has no effect on cell free extracts.²⁰⁴ It is also reported to inhibit protein synthesis and desoxyribonucleic acid synthesis at levels which do not affect respiration or fermentation.²⁰⁵ As more data accumulate on the specific mode of action of the individual antibiotics, one may expect to see a characteristic behavior of each of them. Their properties of greatest interest are their range of harmful action to microbial cells that is not attended by serious disturbance of the cells of the host.

4. INTERACTION OF ANTIBIOTICS: SYNERGISM AND ANTAGONISM

The complexities and ramifications of the action of an antibiotic upon a variety of organisms include a formidable array of possibilities. Depending upon the concentrations of the drug, and those of interfering substances in the medium, different strains of organisms may be unaf-

fected, stimulated, killed or inhibited in their growth. Moreover, these reactions may be entirely different if the results are determined after 10 hours, or if they are evaluated after 30 hours. Furthermore, the results *in vivo* may be very much different from those obtained *in vitro*.

The problems are multiplied when one attempts to determine the effect of one antibiotic upon another. Theoretically, the relationships are simple: (1) if the antibiotics have no effect on each other, their results are additive; (2) if one antibiotic physically prevents the other from acting, the result is a corresponding interference; (3) if one antibiotic affects the organism in a way which prevents effective action by the other, the result is one of antagonism; and (4) if the combination of the antibiotics is more effective than the sum alone, the phenomenon is one of *synergism*. Jawetz and Gunnison¹⁷⁸ have presented many of the details and problems arising from these interrelationships.

The action of antibiotics is additive when they act independently in the presence of each other, and when the number of organisms affected is equivalent to those contained in their combined antimicrobial spectra for the concentration used. The action of antibiotics is synergistic when the total number of organisms affected is materially greater than the sum of those for each antibiotic separately at that particular concentration. Of course, synergistic action can occur only when both compounds are present simultaneously. It is not seen when the antibiotics are added consecutively. Synergism is expected only when there is overlap in the antimicrobial spectra of the compounds under consideration. Antibiotic antagonism is seen when the combined effect of two antibiotics is less than the effect of either one alone (sometimes such antagonism is termed interference),¹⁴⁰ when consideration is given to their overlapping spectra. An antibiotic may act antagonistically toward another antibiotic *in vitro*, but Eagle and Saz conclude¹³² that this relationship may be disregarded *in vivo*. However, a synergism may be of vital importance *in vivo* in diseases such as bacterial endocarditis.¹⁷⁸

Klein and Scharr¹⁴⁰ suggest that antibiotics to which organisms become resistant are the ones which may act synergistically. When no resistance to the agent was seen, the compound did not act synergistically and sometimes did act antagonistically to the other antibiotics. When antibiotics act synergistically, they may be used at 1/3 to 1/20 (sometimes as low as 1/1000 for streptomycin) of the minimum inhibitory concentration. When antibiotics are tested for their antagonistic effect, they must be present in full inhibitory concentration.¹⁴¹ Inactive analogues or degradation products of antibiotics have no part in synergism.

Synergism has been noted frequently with penicillin and sulfonamides, which are more effective in combination than they are separately. They

apparently act in different systems, since little or no cell division occurs in penicillin bacteriostasis, while several generations occur in the presence of sulfonamides.⁹¹ Eagle and Saz¹³² review several papers wherein sub-sterilizing doses of streptomycin rapidly killed organisms which were subjected to low doses of penicillin.

It should be noted that the effect of one antibiotic upon another is not constant, but depends upon the organism being investigated.¹⁴⁰ Bactericidal antibiotics such as penicillin, streptomycin, bacitracin, neomycin and sometimes polymyxin are frequently synergistic, and are often indifferent, but are never antagonistic to each other; while antibiotics which are primarily growth-inhibitory in action are not found to be synergistic or antagonistic to each other.¹³⁹ The latter group include carbomycin, chlortetracycline, chloramphenicol, erythromycin, oxytetracycline, and sulfonamides. Chlortetracycline has been found to inhibit penicillinase formation by staphylococci,¹⁷⁷ an excellent possibility of synergism!

Bacteriostatic compounds which slow the growth of organisms might be expected to inhibit the action of bactericidal drugs which depend upon active metabolism or reproduction for their action. This is the usual situation in antagonism. The antagonistic compound must interfere with a reaction which prevents the cell metabolism from maturing to the condition required for effective action of the second antibiotic. Antagonism occurs when antibiotics are used in low concentrations *in vitro*. When an antibiotic such as cephalosporin P or helvolic acid acts bacteriostatically by extending the lag phase of growth, it may be expected to retard the action of a bactericidal compound which functions most efficiently during reproduction of the cell. Thus helvolic acid is antagonistic to penicillin¹¹³ and cephalosporin P₁ is antagonistic to cephalosporin N. The characteristics of cephalosporin N induce the formation of penicillinase by the cells.¹¹⁴ Biologically-related compounds can be antagonistic by inducing resistance. Thus, staphylococci which have acquired resistance to penicillin can be resistant to bacitracin.¹¹⁵

Antagonistic pairs include penicillin-chloramphenicol, penicillin-tetracyclines, streptomycin-chloramphenicol, streptomycin-tetracyclines, chloramphenicol-neomycin, chloramphenicol-bacitracin, and bacitracin-oxytetracycline.¹⁷⁴

The fact that one or more vitamins, amino acids, adenosine triphosphate (ATP) or natural products often prevent or counteract the action of an antibiotic (see review by Eagle and Saz¹³² for example) would show the antibiotics to be competitive antagonists in specific enzymatic systems. While these reactions are important under the conditions of the experiment, one may suggest they are not necessarily the cytocidal

reactions which make a chemotherapeutic agent. The cytocidal reaction needs to be known by more than the isolated characteristics of an inhibition index, or by their competitive or non-competitive character, or by the nature of their counteracting agents, etc. That is, it is important because it is linked in sequence to bacteriostosis or death. The work of Parks on penicillin presents an outstanding example wherein an initial observation was brought to significance by linking it in sequence to morphological observation of the moribund cell.

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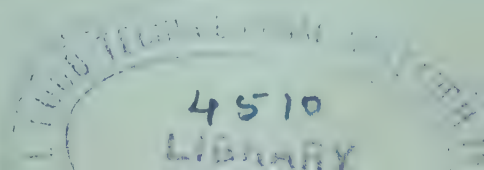
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CHAPTER II

THE CHEMISTRY OF ANTIBIOTICS

BY PETER P. REGNA

A. INTRODUCTION

The isolation of a vast number of antibiotic substances, produced principally by fungi, actinomycetes and bacteria and less commonly from algae, lichens, higher plants, animals, and man, seems, at times to diminish the importance of this particularly difficult field of science. The frequency with which new microbial metabolites are discovered is out of all proportion to the number of those relatively few substances found to meet the requirements set for a satisfactory and useful antibiotic.

Vigorous efforts, however, have gone into antibiotic research since World War II, when the production and possible synthesis of penicillin were highly important to the war effort. Since 1941, we have witnessed many brilliant achievements in microbiology, biochemistry, and organic chemistry by workers in the field of antibiotics.

This feverish activity which today makes possible the discovery of unique antibiotics tends to overshadow the concepts of antibiotic action, which can be traced as far back as 1877 to Pasteur and Joubert.¹ These investigators concluded from experiments that certain aerobic, non-pathogenic organisms interfered with the growth of anthrax bacilli in soils. However, the concept of biologic competition crystallized more recently with the notable discovery of penicillin by Fleming² in 1929, and the work of Dubos³ on gramicidin and tyrothricin in 1939.

The historical background and comprehensive review of the whole antibiotic field up to 1948 has been ably and painstakingly covered in "Antibiotics," by Florey, Chain, Heatley, Jennings, Sanders, Abraham, and Florey.⁴ In addition, a number of reviews on the chemistry of selected antibiotics have appeared in recent years.^{5, 6, 7, 8, 9, 10, 422, 561}

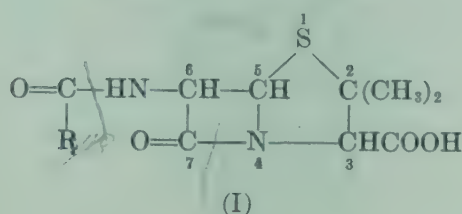
This chapter reviews the chemistry of antibiotics currently used in the treatment of many types of human infections, and those which have attained some stature in non-medical fields. With not too many exceptions, antibiotics that promote growth response in animals, improve plant health, and preserve foods are those of interest in maintaining public health. In order to fill out what otherwise might have been one-sided


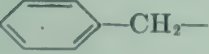
accounts of certain antibiotic developments, this author has added to certain sections of this chapter related and pertinent information which bears on antibiotics of lesser importance.

B. PENICILLIN

The original strain used for the production of penicillin yielded at least six closely related penicillins (Table 2-1) and a series of mold pigments. Several of these colored crystalline substances gradually deteriorated after standing in our laboratories for a period of years.

TABLE 2-1
THE PENICILLINS



Penicillin	Side Chain R	Potency in Oxford units of sodium salts
(G) Benzyl <i>Peni</i>	 —CH ₂ —	1,667
(X) p-Hydroxybenzyl	HO—  —CH ₂ —	900
(F) 2-Pentenyl	CH ₃ CH ₂ CH=CHCH ₂ —	1,600
3-Pentenyl	CH ₃ CH=CHCH ₂ CH ₂ —	
(Dihydro F) n-Amyl	CH ₃ CH ₂ CH ₂ CH ₂ CH ₂ —	1,500
(K) n-Heptyl	CH ₃ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ —	2,300

The penicillins produced in media free of precursors differ only with respect to the side chains R, which are attached to a common nucleus (I). All these types of penicillin exhibit high optical activity. They contain a common acid function of about pK 2.8 and are equally susceptible to decarboxylation, or to rupture of the β -lactam ring into biologically inactive derivatives. Benzyl penicillin (G) is the leading product of modern fermentation methods and is the most important form of the antibiotic on the market.

The chemical structure of penicillin was the subject of an intensive Anglo-American collaborative study during World War II. In their early efforts, its investigators, working under security regulations imposed by both Governments, were handicapped both by the extremely small

amounts of antibiotic obtained from low potency fermentations, and by the meager recovery yields. In addition, considerable confusion arose from results of mild acid hydrolysis of penicillin. The British obtained a crystalline penillic acid with the empirical formula, $C_{14}H_{20}N_2O_4S$, and the Americans under identical conditions isolated a substance with the formula $C_{16}H_{18}N_2O_4S$. This discrepancy was resolved by the fermentation studies of Moyer & Coghill.²⁰⁷ They showed that different species of penicillin were produced by *Penicillium notatum*, according to the nature of the medium. Thus, the British penicillin was designated penicillin F, and that produced in the United States, penicillin G. Both penillic acids (F and G) (VIII) give the same penicillamine (V) on acid hydrolysis. The penilloaldehyde (VII), however, derived from penicillin F, yields β -hexenoylaminoacetaldehyde (II), and that from penicillin G gives phenylacetamidoacetaldehyde (III).¹¹



β -Hexenoylaminoacetaldehyde

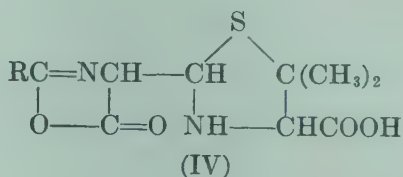
(II)



Phenylacetamidoacetaldehyde

(III)

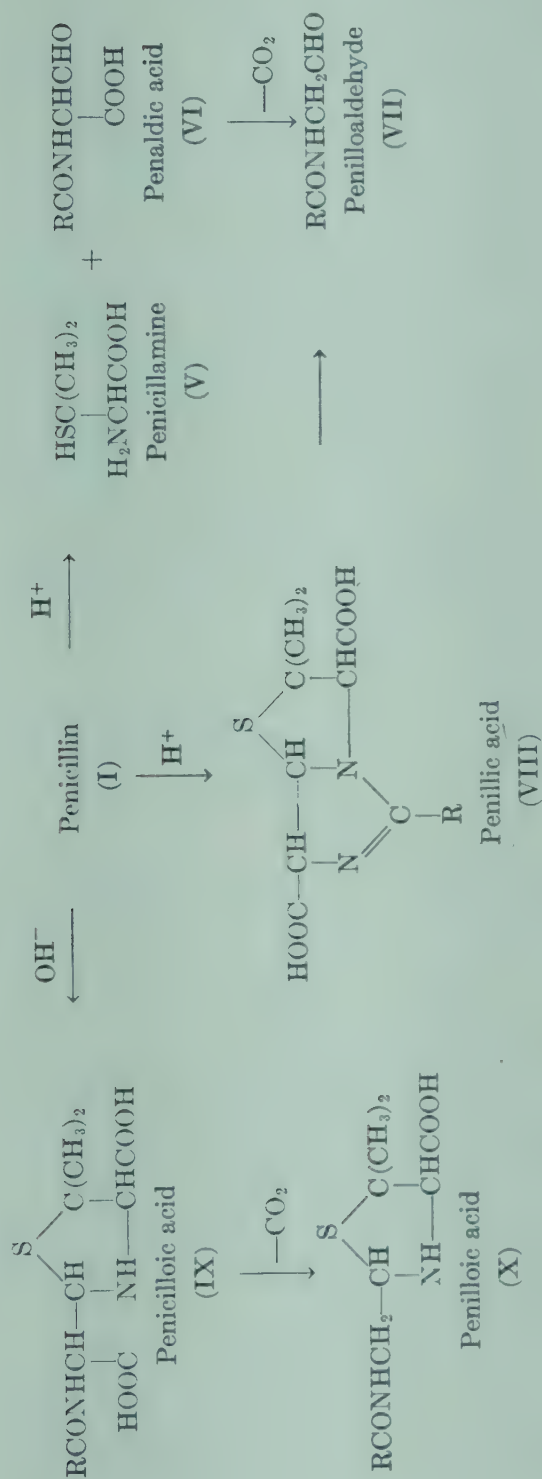
Structural interpretations led to several proposed formulas for the antibiotic. For a time both American and British groups focused their attention on the oxazolone-thiazolidine formula (IV). Because the basic group in IV could not be detected, the British chemists and the Merck group in America proposed the β -lactam (I) structural variant as an



(IV)

alternative. Conclusive support for the β -lactam structure was provided by electron density projections¹² and infrared absorption measurements.^{11,12}

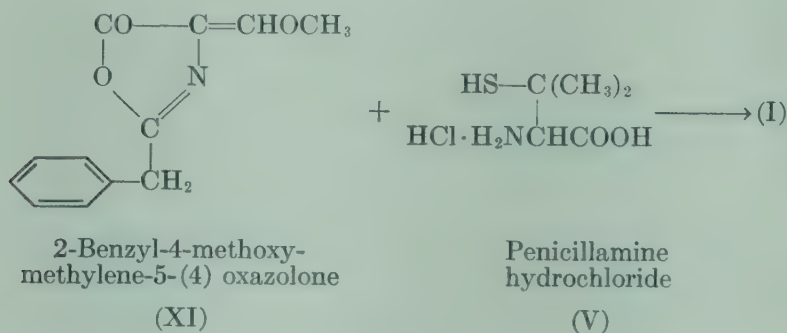
It was some time, however, before the reactions in Scheme 1 were completely understood. In acid solution, penicillin is readily cleaved into an amino acid, penicillamine (V) (β -thiol-*D*-valine) common to all penicillins, and a second fragment, penaldic acid (VI), which differs with



Scheme 1

the nature of the alkyl group R in the penicillin. The penaldic acids (VI) are unstable and readily lose carbon dioxide to give the corresponding penilloaldehydes (VII). Mild acid hydrolysis of I yields penillic acid (VIII), which can be degraded farther to (VII). Alkaline hydrolysis, on the other hand, by opening the β -lactam ring of penicillin, yields penicilloic acid (IX); this substance readily loses carbon dioxide to give penilloic acids (X). Acid hydrolysis also cleaves penicilloic acids (IX) into penicillamine (V) and penaldic acids (VI).¹¹

Many attempts have been made to synthesize penicillin,^{13,14,15,16,17} but nearly all results have been disappointing. The one exception is the distinguished work of Professor J. C. Sheehan and K. R. Hénerly-Logan, who accomplished the first rational synthesis of a natural penicillin.¹⁸ However, early in the course of research, the Merck group, and later in 1944 the Oxford group, based their approach on the incorrect assumption that penicillin had the oxazolone-thiazolidine formula (IV).¹⁹ The reaction involved the condensation of *D*-penicillamine hydrochloride (V) with 2-benzyl-4-methoxymethylene-5-(4)-oxazolone (XI) (Scheme 2) to yield



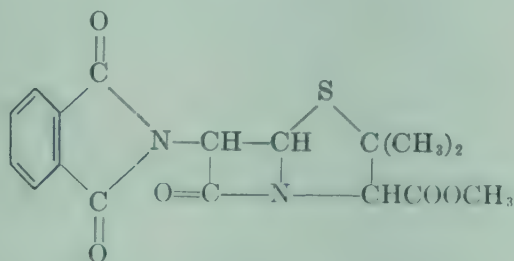
Scheme 2

a product with a very low order of biologic activity. The presence of benzyl penicillin (I) in a mixture containing 0.1 percent penicillin activity was established unequivocally by du Vigneaud, Carpenter, Holley, Livermore, and Rachele.²⁰ They isolated the antibiotic from the reaction mixture by Craig countercurrent distribution²¹ and crystallization of the triethylammonium salt.^{19,20}

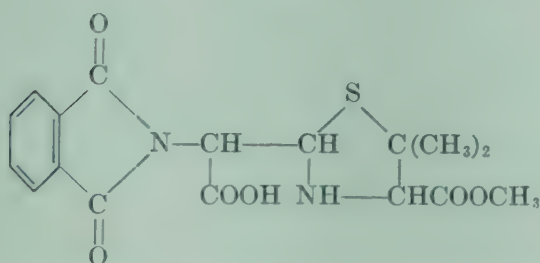
More recently Sheehan and his co-workers^{13,22,23} prepared the fused thiazolidine- β -lactam, methyl-6-phthalimidopenicillanate (XII),* which has the complete structure of the natural penicillins (configuration unassigned) except for the substitution of an ester group and a phthalimido group for the usual acylamino group. This synthesis has depended upon

* For nomenclature, see reference 13.

the cyclization of the α -isomer²³ of the three stereoisomeric modifications of the key intermediate XIII. Structure XIII^{22,23,24} is so constituted as

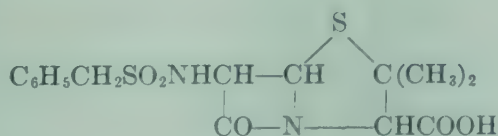


Methyl-6-phthalimidopenicillanate
(XII)



4-Carbomethoxy-5,5-dimethyl- α -phthalimido-2-thiazolidineacetic acid
(XIII)

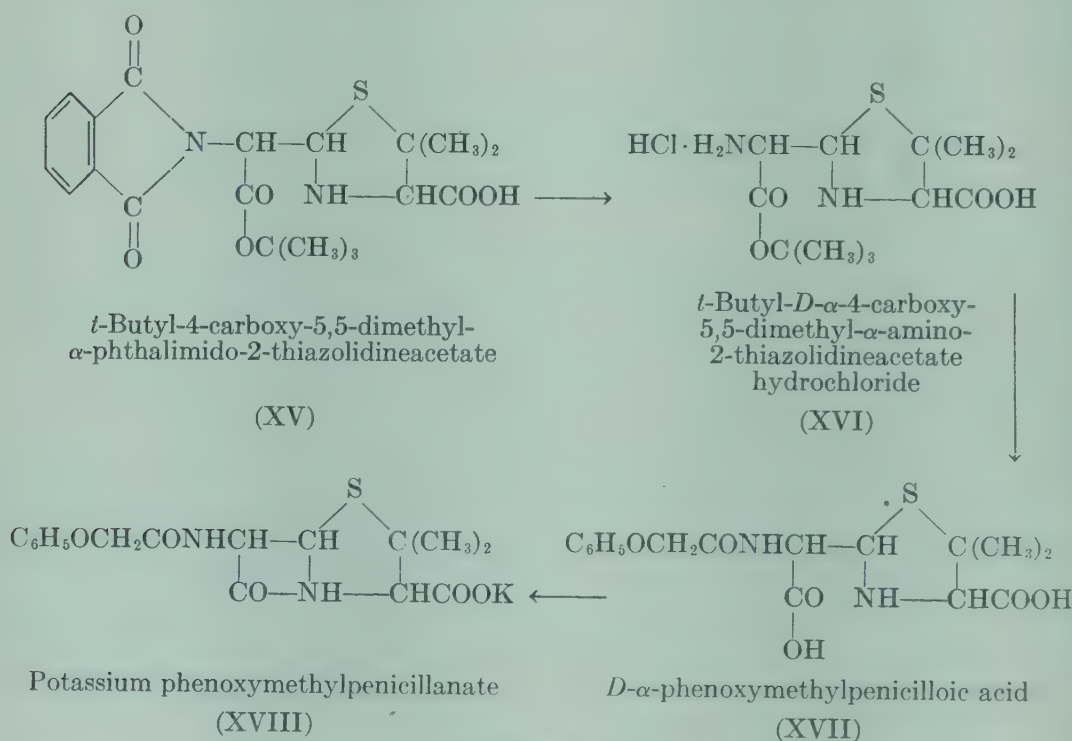
to prevent the formation of a five-membered azlactone ring with the amide nitrogen of a penicilloate (IX). Presence of the phthaloyl blocking group in the precursor (XIII) blocks azlactonization, and thus the ring-closure to the β -lactam (XII) becomes the alternative reaction.^{22,23,24} In presenting this approach, Sheehan and Cruickshank reported in 1956 on the total synthesis of methyl *DL*-benzylpenicillanate sulfone,^{25,26} a substituted penicillin closely related to natural benzylpenicillin. A further advance was made by Sheehan and Hoff²⁷ with the preparation of the crystalline tertiary amine salt of the acid-stable, biologically active sulfonyl analog (XIV) of benzylpenicillin. This analog differs from the natural antibiotic only in the presence of a sulfur atom instead of a carbon atom in the side-chain amide.



6-Benzylsulfonamidopenicillanic acid
(XIV)

Subsequently, Sheehan and Hénerly-Logan completed the synthesis of a biologic penicillin,¹⁸ phenoxymethylpenicillin (penicillin V) (XVIII),²³ thereby overcoming the hitherto insurmountable sensitivity of the β -lactam. Rupture of the β -lactam was avoided by the resourceful cyclization of *D*- α -phenoxymethylpenicilloic acid (XVII) in *N,N'*-dicyclohexylcarbodiimide-dioxane-water at 25° C for twenty minutes.

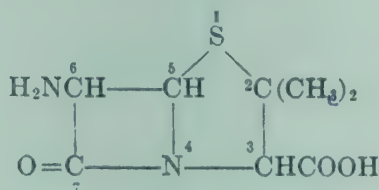
The synthesis was carried out according to Scheme 3. *D*-penicillamine (V) was condensed with *t*-butyl phthalimidomalonaldehyde to give the compound related to XIII—*t*-butyl 4-carboxy-5,5-dimethyl- α -phthalimido-2-thiazolidineacetate (XV).²⁴ The next intermediate, *t*-butyl *D*- α -4-carboxy-5,5-dimethyl- α -amino-2-thiazolidineacetate hydrochloride (XVI), was obtained by hydrazinolysis of XV. Phenoxyacetyl chloride and triethylamine converted XVI into α -*t*-butyl *D*- α -phenoxymethylpenicilloate, from which *D*- α -phenoxymethylpenicilloic acid (XVII) was obtained by cleavage of the *t*-butyl ester with dry hydrogen chloride. Treatment of XVII with *N,N'*-dicyclohexylcarbodiimide in dioxane-water gave 12% yield of phenoxymethylpenicillanate (XVIII) crystallized as



Scheme 3

the potassium salt. The fermentation and synthetic potassium salts were shown to be identical by microbiologic assay, optical rotation, infrared spectrum, and melting point comparisons. The method is certain to provide new penicillins not accessible by biosynthesis and could conceivably make available a form resistant to penicillinase inactivation.

Similar opportunities are afforded by the isolation of 6-aminopenicillanic acid (XVIIIa) by Rolinson, Doyle, Batchelor, and Nayler (565). The parent compound will provide a framework for new types of penicillin by acylation of the 6-amino group in XVIIIa.



6-Aminopenicillanic acid
(XVIIIa)

The instability of penicillin has been the greatest obstacle to efforts of organic chemists to vary the molecule. For this reason, emphasis has been put on biosynthesis of penicillins^{29,30} by fermentation in the presence of substituted amides whose acyl groups are incorporated into the penicillin molecule. This process is limited to alterations of the R-group of I and does not vary the main structural features of the penicillin molecule. A number of these biosynthetic penicillins are known, but relatively few find even moderate use because, in some cases, they have not been evaluated adequately in humans. For example, phenoxymethylpenicillin (penicillin V) was prepared by Behren²⁸ and his co-workers in 1947, but its stability in gastric acids and its ability to produce more consistent blood levels in humans were not recognized until 1953.^{31,32}

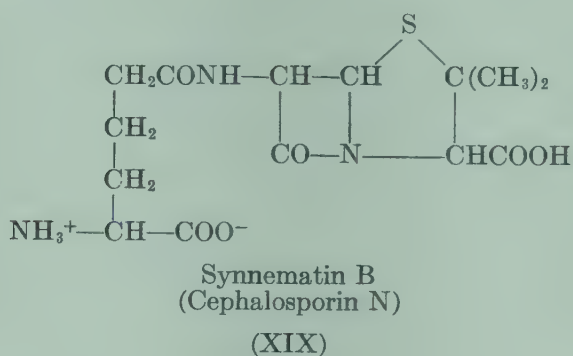
Chemical modifications have been made in certain of these biosynthetic penicillins, but they are limited to: (1) the R-groups in those biosynthesized penicillins with functional groups, such as the OH-group in p-hydroxybenzylpenicillin; (2) the preparation of esters formed by reacting diazoalkanes with the free acid of penicillin; and (3) formation of the amide.³³ The organic chemist has been more successful in devising a series of penicillin salts which have low solubility in water or body fluids, decreased allergenic reactions, and reduced pain at the site of injection.

While the procaine salt^{34,35} continues to be the most widely used repository preparation of penicillin G, several other salts of penicillin are finding application in current medical practice³⁶ viz., N,N'-dibenzylethylenediamine dipenicillin G (benzathine penicillin G)³⁷; 2-chloroprocaine allylmercaptomethyl penicillin (chloroprocaine penicillin O)^{38,39}; L-N-methyl-1,2-diphenyl-2-hydroxyethylamine penicillin G (L-phenamine penicillin G)⁴⁰; the hydroiodide of diethylaminoethyl ester of penicillin G⁴¹; the N,N-dimethyl-N'-benzyl-N'-(α -pyridyl) ethylenediamine penicillin G (pyribenzamine penicillin)⁴²; and dibenzylamine penicillin G.⁴³

Synnematin (Cephalosporin N). In a search for microorganisms

capable of producing useful antibiotic substances, Gottshall, *et al*, in 1951^{44,45} found that a strain of *Cephalosporium salmosynnematum* elaborated a mixture of active components, one of which was found to be a new penicillin called synnematin B.⁴⁶ The production, recovery, and separation of the antibiotic into synnematin A and B was achieved by Olson, Jennings, Pisano, and Junek.^{46,47,48} The B-component of this mixture has a low toxicity and is effective in the treatment of experimental *Salmonella* infection in mice and chicks⁴⁸ and in the treatment of typhoid-infected patients.⁴⁹

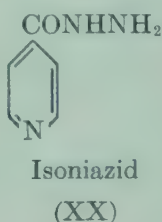
It is not uncommon in antibiotic research to find investigators in two or more independent laboratories engaged in the study of the same antibiotic. In 1951, Burton and Abraham⁵⁰ reported on the isolation of antibiotics from a species of *Cephalosporium*. From this mixture of antibiotic substances cephalosporin N was isolated and characterized as a new type of penicillin by Abraham, Newton, Crawford, Burton, and Hale.⁵¹ The antibiotic differs strikingly from the common penicillins in its hydrophilic character and its antibacterial activity, but like penicillin it is inactivated by the enzyme penicillinase.^{51,52} Acid hydrolysis of a highly purified amorphous barium salt produced penilloic acetate from which penicillamine (V) was obtained on treatment with mercuric acid. More vigorous hydrolyses of the penilloic acid liberated *D*- α -aminoadipic acid.⁵³ From these and other studies of structure it was concluded that cephalosporin N is *D*-4-amino-4-carboxy-n-butyl penicillin (XIX),⁵⁴ and is identical with synnematin B.^{55,422}



C. STREPTOMYCIN AND RELATED ANTIBIOTICS

The extraordinary effect of penicillin against most gram-positive cocci and other bacteria stimulated the search for antibacterials inhibitory against gram-negative bacilli and the acid-fast microorganisms. In several laboratories, particular attention was focused on the production of antibiotic substances from actinomycetes. As a result of one such

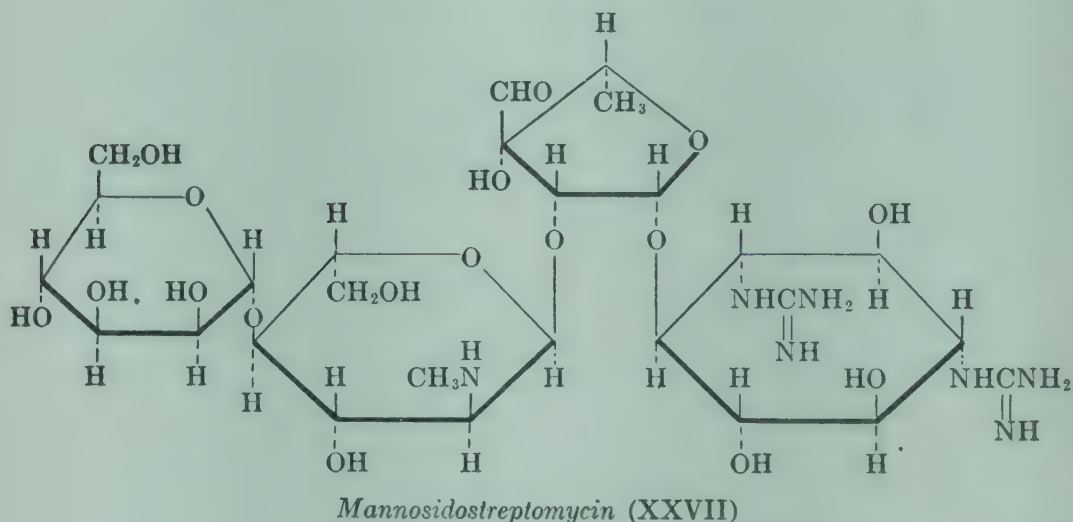
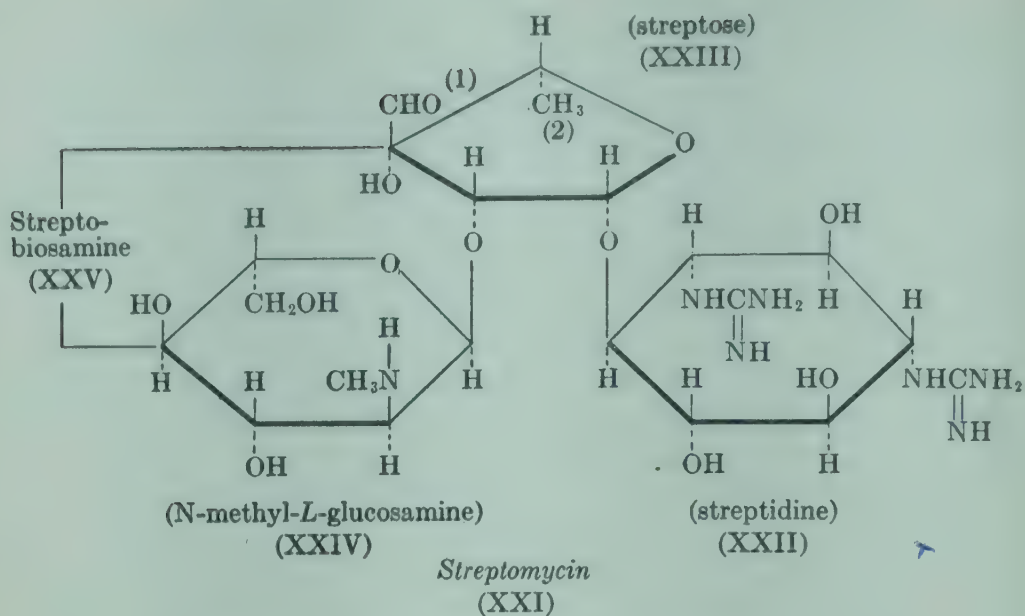
program, streptothricin was isolated in 1942 by Waksman and Woodruff.⁵⁶ Lasting several years, the search involved isolation and examination of more than 10,000 microorganisms before streptomycin was isolated by Schatz, Bugie, and Waksman from two *Streptomyces griseus* strains.⁵⁷ The antimicrobial spectrum of streptomycin was found to differ greatly from that of penicillin; it has a characteristic effect against gram-negative microorganisms⁵⁷ and a high degree of activity against mycobacteria, particularly human pathogens.⁵⁸ Interest in a potential antituberculous agent was aroused quickly. In retrospect, this optimism was highly justified, since even now the only drug of comparable importance in tuberculosis therapy is isoniazide (XX), the hydrazide of isonicotinic acid, the activity of which was not demonstrated until 1952.



Streptomycin. While streptomycin was undergoing purification, characterization, and structural studies by several groups,^{59, 60, 61, 62} the effects of crude streptomycin were being observed in experimental animals infected with *Pasteurella tularensis*,⁶³ *Salmonella schottmülleri*,⁶⁴ *S. pul-lorum*,⁶⁵ *Mycobacterium tuberculosis*,⁶⁶ etc. and even in the treatment of human tuberculosis.⁶⁷

Chemically, streptomycin is an optically active, triacidic base with an empirical formula $\text{C}_{21}\text{H}_{39}\text{N}_7\text{O}_{12}$. A variety of organic and inorganic salts of the antibiotic have been prepared; the sulfate, the trihydrochloride, and the calcium chloride double salt, $(\text{C}_{21}\text{H}_{39}\text{N}_7\text{O}_{12} \cdot 3\text{HCl})_2 \cdot \text{CaCl}_2$, are important. Of great aid in the purification of crude streptomycin⁶⁸ are such crystalline salts as the p-(3-hydroxy-1-naphthylazo)-benzene sulfo-nate,^{60, 69} the naphthalene β -sulfonate⁷⁰ and the calcium chloride double salts.⁷¹ In addition, a series of therapeutic salts have been prepared, for example, streptomycin 2-hydroxy-4-aminobenzoate.⁷²

The very difficult task of elucidating the structure and configuration of the antibiotic was carried out principally by K. Folkers, O. Wintersteiner, H. E. Carter, M. L. Wolfrom, and their respective associates. Their work has been the subject of several excellent reviews.^{73, 74, 75, 76} Streptomycin (XXI) is composed of three moieties (Scheme 4): streptidine (XXII), streptose (XXIII), and N-methyl-L-glucosamine (XXIV) joined by glycosidic bonds.⁷⁷ On hydrolysis with acid, the weaker glyco-



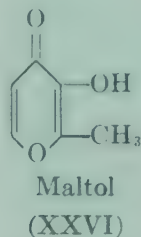
Scheme 4

sidic bond in streptomycin^{77,78} between streptidine and streptose is split to give streptobiosamine (XXV)^{79,80,81} and streptidine (XXII), a *meso* form of 1,3-diguanido-2,4,5,6-tetrahydrocyclohexane,^{82,83,84} the structure having been confirmed by synthesis.^{85,86} Structure of the nitrogen-containing disaccharide (XXV) was determined by a study of the hydrolysis products of the methyl ether and the ethyl thioether formed by the action of hydrogen chloride in methanol and ethylmercaptan, respectively. The hydrolysis of methylstreptobiosaminide gave N-methyl-L-glucosamine

(XXIV),⁸⁷ the non-natural form of this amino sugar. Its structure also has been established by synthesis.⁸⁸

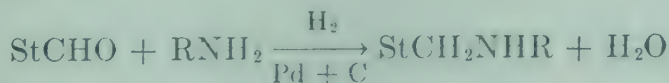
Because of its instability, the determination of the structure of the hitherto unknown monosaccharide, streptose (XXIII), presented considerable difficulties, but was finally shown to be 3-C-formyl-5-deoxy-*L*-lyxose, a pentose containing the reactive aldehyde group associated with the streptomycin molecule.^{80,81,89,90,91,92}

The aldehyde group, during hydrolysis of streptomycin in alkali, becomes involved in the formation of maltol (XXVI). The reaction is the



basis of a quantitative method for the estimation of streptomycin.⁹³ Presence of the aldehyde group was demonstrated in streptomycin by oxidation with bromine to an acid, and by the formation of oxime and semicarbazone derivatives, all of which are biologically inactive.⁷⁹ Certain derivatives such as the crystalline streptomycylidene isonicotinyl hydrazone sulfate⁹⁴ derive their activity by hydrolysis in body fluids to streptomycin and isoniazid.⁹⁵ Reduction of the aldehyde to an alcohol group, however, by hydrogenation with platinum⁹⁶ or nickel⁹⁷ catalysts gives dihydrostreptomycin (XXVI). Dihydrostreptomycin is produced also by a species of microorganisms named *Streptomyces humidus*.⁵⁵³

Dihydrostreptomycin possesses a biologic activity comparable to streptomycin, does not respond to carbonyl reagents, does not give the maltol (XXVI) reaction, does not form a calcium chloride double salt and, unlike streptomycin, is relatively stable to alkali.⁹⁶ It can be crystallized as the base⁹⁸ and as the sulfate.⁹⁹ The sulfate melts at 255-265° C and has a value of $[\alpha]_D^{25} - 88^\circ$ (in water). Winsten, Jarowski, Murphy, and Lazier¹⁰⁰ prepared a number of active *N'*-alkylstreptomycylamines by reductive alkylation,^{100,101} symbolized by,



in which St represents streptomycin minus the aldehyde group, and R denotes an alkyl or aryl group. These streptomycin derivatives are somewhat more toxic than the parent substance. A compound, bis (α -hydroxystreptomycyl) amine, which exhibits a toxicity in mice 100

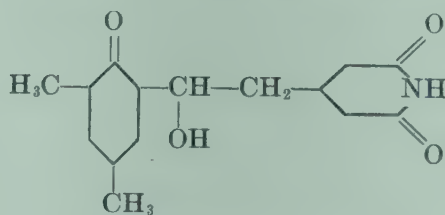
times greater than pure streptomycin, was obtained by Solomons and Regna¹⁰² as the result of interaction of streptomycin with ammonium chloride in aqueous methanol.

Mannosidostreptomycin, *Hydroxystreptomycin* and *Actidione*. *Streptomyces griseus* produces a number of antibiotics in addition to streptomycin. Those mentioned here are of somewhat greater interest by reason of their chemical structures or unusual biologic properties.

Mannosidostreptomycin (XXVII) was isolated from streptomycin cultures by Fried and Titus^{103,104} using Craig countercurrent distribution techniques.²¹ Fried and Stanley^{105,106} showed that degradation of XXVII gave rise to derivatives of streptidine (XXII), streptobiosamine (XXV), and *D*-mannose, and further that the streptomycin moiety is attached glycosidically to *D*-mannose through C-4 of the *N*-methyl-*L*-glucosamine fragment.¹⁰⁷ In addition, Peck, Hoffhine, Gale, and Folkers showed that in mannosidostreptomycin, the mannosidostreptobiosamine is attached to C-4 of the streptidine, just as streptobiosamine (XXV) is attached to streptidine (XXII) in streptomycin.¹⁰⁸ This antibiotic (XXVII) can be hydrogenated, under conditions used for streptomycin, to yield dihydromannosidostreptomycin.¹⁰⁵ Cultures of *S. griseus* which produce streptomycin contain an enzyme able to convert mannosidostreptomycin to streptomycin.¹⁰⁹

Hydroxystreptomycin (XXVIII)¹¹⁰ was isolated from *Streptomyces griseocarneus* by Benedict, Stodola, Shotwell, Borud, and Lindenfelser.¹¹¹ It differs from streptomycin only in the replacement of a hydrogen atom by an oxygen atom in the streptose portion of the molecule.¹¹²

Cycloheximide (XXIX) (Actidione) β -[2-(3,5-dimethyl-2-oxycyclohexyl)-2-hydroxyethyl] glutarimide differs markedly from streptomycin in biologic activity and chemical structure.^{113,114} The antifungal antibiotic was isolated by Whiffen, Bohonos, and Emerson¹¹⁵ from streptomycin cultures of *S. griseus*. Brown and Hazen¹¹⁶ demonstrated that

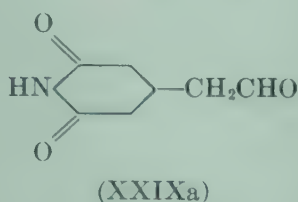


Cycloheximide (XXIX)

Streptomyces noursei produces nystatin (fungicidin) in the mycelium and actidione in the culture filtrates. Cycloheximide crystallizes in colorless plates, m.p. 115°–116° C; $[\alpha]_D^{25} - 2.8^\circ$ (in methyl alcohol).¹¹⁷ This simultaneous biochemical synthesis of streptomycin and cycloheximide

is an example of the well-recognized ability of many microorganisms to produce two or more substances with entirely distinct chemical and antimicrobial properties.

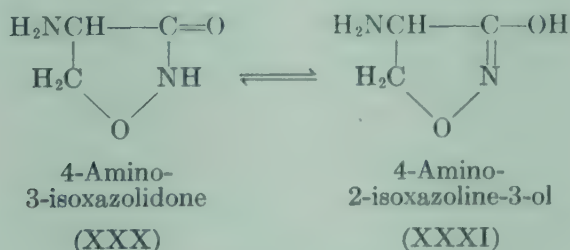
Assignment of structure XXIX has not yet been verified by total synthesis. Attempts have been made to condense the glutarimide portion (XXIXa) of the actidione molecule with (+)-2,4-dimethylcyclohexa-



none⁴⁷⁴ in the expectation that this reaction might lead to a total synthesis of actidione. Djerassi and co-workers⁵¹¹ have shown that the methyl groups in the ketone moiety have a *cis* configuration.

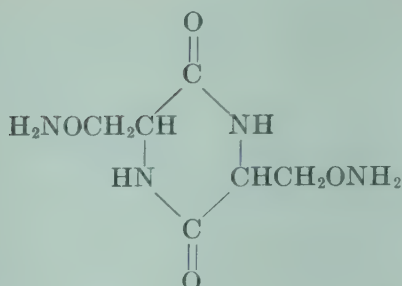
Cycloserine is another potent antituberculous antibiotic.^{118,119} Several different groups of investigators demonstrated the ineffectiveness of cycloserine in mouse¹¹⁸ and guinea pig tuberculosis,¹²⁰ notwithstanding its *in vitro* activity. In fact, the antibiotic might well have been abandoned because it exhibited limited effects on screening in animals. Because it is virtually non-toxic in animals, however, Epstein, Nair, and Boyd¹¹⁹ tested it in humans and found cycloserine an effective drug in treatment of pulmonary tuberculosis.

The antibiotic was isolated independently by Shull, Routien, and Finlay as a metabolic product of *Streptomyces lavendules*^{121,122} by Harned, Hidy, and Kropp-La Baw¹²³ as a product of *Streptomyces orchidaceus*, and by Harris, Ruger, Reagan, Wolf, Peck, Wallick, and Woodruff¹²⁴ from culture filtrates of *Streptomyces garyphalus*. The antibiotic has been shown to be *D*-4-amino-3-isoxazolidone (XXX)^{125,126} and can be represented as indicated in Scheme 5. In aqueous solution, it exists as a dipolar ion.



Scheme 5

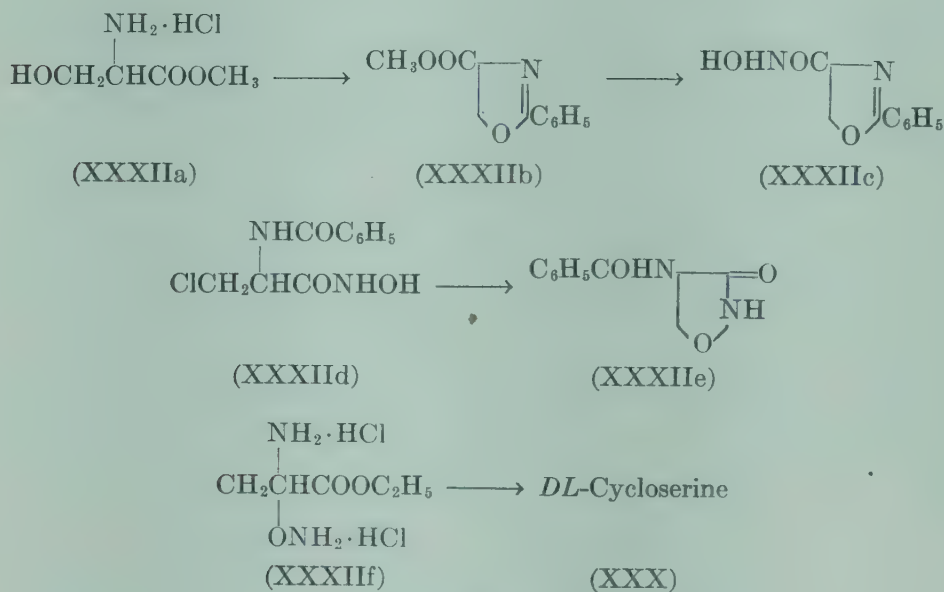
On standing in solution, cycloserine dimerizes to 2,5-bis-(aminoxymethyl)-3,6-diketopiperazine (XXXII).¹²⁶



2,5-Bis-(aminoxymethyl)-3,6-diketopiperazine (XXXII)

D-4-amino-3-isoxazolidone is obtained as colorless crystals, m.p. 154° – 155° ; $[\alpha]_D^{25} + 116^{\circ}$ (in water). It forms a stable silver salt of composition $C_3H_5N_2O_2Ag$,¹²⁵ and forms also calcium, barium, magnesium, and sulfate salts.¹²⁶ It is relatively stable to alkali, but in acid it is degraded readily to hydroxylamine and serine as the major products.

Cycloserine has been synthesized, as outlined in Scheme 5a, by Stammer, Wilson, Spencer, Bachelor, Frederick, Holly, and Folkers^{127,476} starting with *DL*-serine methyl ester hydrochloride (XXXIIa). The



Scheme 5a

ester was converted on reaction with ethyl iminobenzoate, into *DL*-2-phenyl-4-carbomethoxy-2-oxazoline (XXXIIb). On treatment of XXXIIb with hydroxylamine and sodium methoxide, 4-carbohydroxamido-2-phenyl-2-oxazoline (XXXIIc) was obtained. Heating XXXIIc in dioxane containing hydrogen chloride formed α -benzamido- β -chloropropionohydroxamic acid (XXXIIId) which was cyclized to 4-benzamido-3-isoxazolidone (XXXIIe) by aqueous alkali. Boiling ethanol saturated

with hydrogen chloride removed the benzoyl group and opened the isoxazolidone ring of XXXIe giving the dihydrochloride of *DL*- β -aminoxyalanine ethyl ester (XXXIf). This ester cyclized rapidly to *DL*-cycloserine (XXX) on treatment with aqueous potassium hydroxide. The racemate was resolved with *D*-tartaric acid to give *D*-4-amino-3-isoxazolidone-*D*-tartrate ($[\alpha]_D^{25} + 41^\circ$), from which the antibiotic (XXX) was recovered and found to be identical with the natural product. By use of *L*-tartaric acid, *L*-4-amino-3-isoxazolidone-*L*-tartrate ($[\alpha]_D^{25} - 40^\circ$) was obtained from the racemate.

Since cycloserine is configurationally related to *D*-serine, a shorter synthesis starting with *D*-serine, was developed by Smrt, Beránek, Sicher, Škoda, Hess, and Šorm⁴⁷⁶ in 1957. The *L*-isomer was prepared from *L*-serine, and the activities of *D*-, *L*-, and *DL*-forms of the antibiotic were compared when tested against *Escherichia coli*. The *D*- and *L*- forms of the antibiotic were found to have comparable activities, but surprisingly less than that exhibited by the racemate. The behavior of the racemate is undoubtedly the first case of synergism produced by stereoisomeric forms of an antibiotic.^{476, 556, 557, 558}

D. CHLORAMPHENICOL

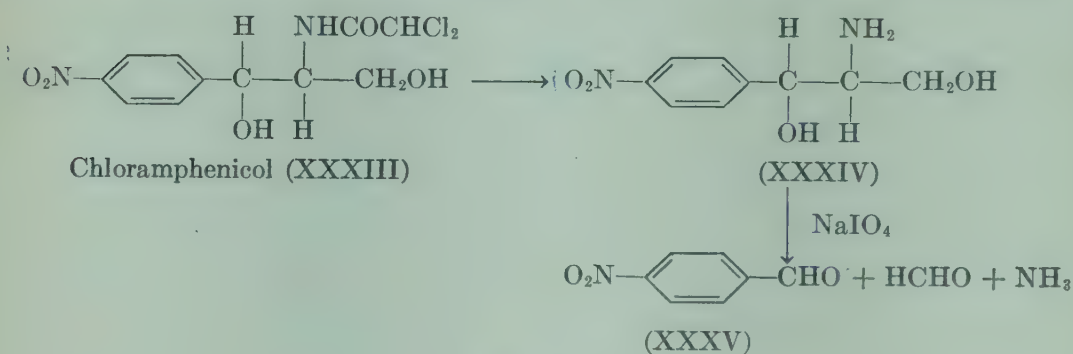
The discovery of chloramphenicol by Burkholder in 1947 established the existence of antibiotics produced by microorganisms isolated from natural sources and effective against a greater variety of pathogenic microorganisms. Such substances are now known as "broad spectrum" antibiotics. However, because of the intensive search for new antibiotics in different laboratories, it frequently happens that independent investigators discover the same antibacterial substance. Thus, chloramphenicol was isolated by Ehrlich, Bartz, Smith, Joslyn, and Burkholder¹²⁸ originally from the elaboration products of a Venezuelan soil organism called *Streptomyces venezuelae*,¹²⁹ and later by Carter, Gottlieb, and Andersen¹³⁰ from a similar organism found in a compost soil in Illinois. Since other strains of actinomycete also produce the antibiotic,¹³¹ it has subsequently been isolated independently several times in pharmaceutical laboratories from culture filtrates of organisms obtained in soils from widely scattered places.

Chloramphenicol inhibits many gram-positive and gram-negative cocci and bacilli, spirochetes, actinomycetes, several Rickettsiae, and certain large viruses.^{128, 132, 133, 134, 135} It was the first antibiotic found to be effective in the treatment of typhoid in humans, even though it possesses only a low order of protection in experimental infections in mice.¹³⁶

Chloramphenicol, D-(—)-threo-2-dichloroacetamido-1-*p*-nitrophenyl-1,3-propanediol (XXXIII) crystallizes in colorless needles having a m.p.

150.5–151.5° C; an optical rotation $[\alpha]_D^{25} + 19^\circ$ (in methyl alcohol); and a solubility of 2.5 mg. per ml. of water at 25°. It is soluble in alcohols, fairly soluble in ether, and insoluble in benzene and petroleum ether. Solutions of the antibiotic in water are faintly acid (pH 5.5),¹²⁹ are sensitive to light or high temperature, and decompose with the formation of chloride ion and other degradation products.¹³⁷ By a series of degradation reactions, physical data, and finally, by synthesis, Rebstock, Crooks, Controulis, and Bartz^{138,139} showed that chloramphenicol has a structure related to *L*-pseudo-norephedrine.¹⁴⁰ A configurational correlation between XXXIII and *D*-serine also has been established.¹⁴¹ The molecule (XXXIII) consists of a 2-acylamidopropanediol side chain containing two asymmetric carbon atoms attached to a *p*-nitrophenyl group. The first suggestions that the antibiotic contained a *p*-nitrobenzene moiety, deduced from its characteristic absorption band at 278 m μ , were received with some doubts, because no microorganism-produced substance with a nitro group had been detected previously,¹⁴² although since then, at least one other antibiotic, azomycin, 2-nitroimidazole has been isolated.⁵⁵⁵

Alkaline or acid hydrolysis of the antibiotic yield dichloroacetic acid and an optically active base (XXXIV) (Scheme 6), exhibiting the proper-

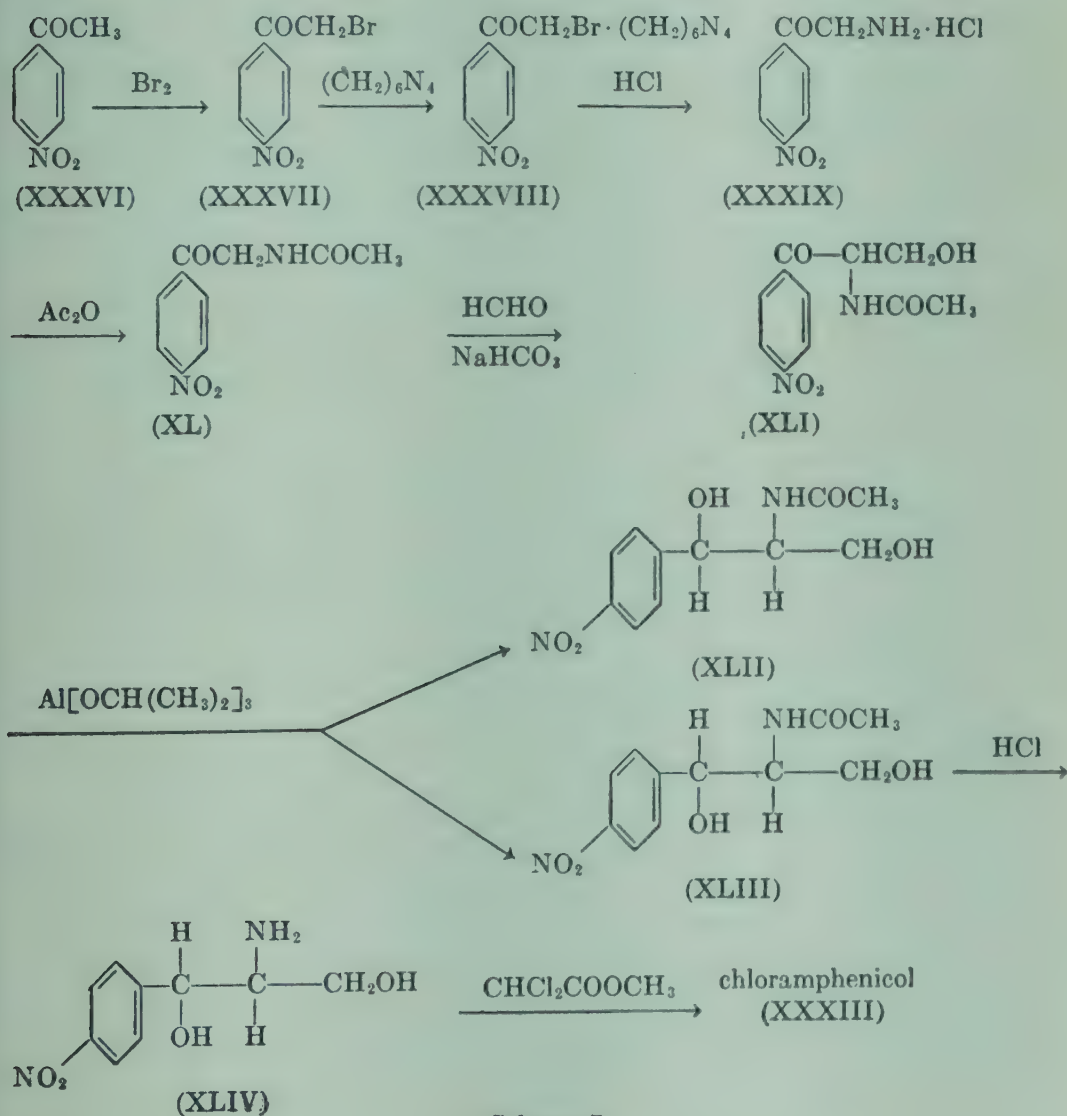


Scheme 6

ties of an amine. Oxidation of the amine with sodium periodate gives *p*-nitrobenzaldehyde (XXXV). Chloramphenicol was reformed by reacting the amine (XXXIV) with methyl dichloroacetate.¹³⁹ The ease with which activity was restored when the two inactive fragments were recombined hastened synthetic approaches which eventually confirmed the deduced constitution of the antibiotic. Synthetic chloramphenicol, identical in chemical and biologic properties with the fermentation product, was obtained by Controulis, Rebstock and Crooks¹³⁸ through appropriate intermediates derived from condensing benzaldehyde with β -nitroethanol¹³⁸ or benzaldehyde with nitromethane.¹⁴³ These syntheses produce the four possible *D*- and *L*-threo and *D*- and *L*-erythro isomers related to XXXIII, three of which are virtually inactive in biologic tests.

The stereochemical configuration of the side chain is specific for antimicrobial action because, of the four possible stereoisomers, only the *D*-threo possesses this activity.^{139,144}

Chloramphenicol is still the only clinically important antibiotic that can be prepared synthetically in competition with fermentation methods.¹⁴⁵ The synthetic process in more general use is based upon a series of reactions which start with *p*-nitroacetophenone.^{145,146} The *p*-nitroacetophenone is prepared by condensing *p*-nitrobenzoyl chloride with the magnesium methoxy derivative of diethyl malonate. The resulting diethyl *p*-nitrobenzoylmalonate is hydrolyzed in dilute acid to yield *p*-nitroacetophenone (XXXVI). The method of synthesis is outlined in Scheme 7, and emphasizes more clearly the stereochemistry of the antibiotic.



Scheme 7

The method can utilize acetophenone alternatively,¹⁴⁷ but usually *p*-nitroacetophenone (XXXVI) is brominated to *p*-nitro- α -bromoacetophenone (XXXVII). Reaction of XXXVII with hexamethylenetetramine forms the salt XXXVIII, which on hydrolysis in alcoholic hydrochloric acid yields *p*-nitro- α -aminoacetophenone as a stable hydrochloride (XXXIX). The amino ketone (XXXIX) is unstable in the free state. Therefore, before proceeding to the next step, XXXIX is acetylated with acetic anhydride to protect the amino group. Hydroxymethylation of the acetylated derivative (XL) with formaldehyde in the presence of sodium bicarbonate yields *p*-nitro- α -acetoamido- β -hydroxypropionophenone (XLI). Since the compound XLI contains an asymmetric center, it occurs in optically active forms; however, synthetic methods produce only racemic mixtures containing equal amounts of both stereoisomers. Reduction of XLI with aluminum isopropoxide by the method of Meerwein-Ponndorf yields XLII and XLIII, each of which now contain two asymmetric centers, thus providing two racemic mixtures, four compounds in all. One set of isomers represented by XLII is composed of the *D*- and *L*-erythro-2-acetamido-1-*p*-nitrophenyl-1,3-propanediol diastereoisomers, and a second set represented by XLIII is the *D*- and *L*-threo racemate. The advantage of this synthetic method resides in almost exclusive formation of the *D*- and *L*-threo racemate and its ease of isolation from the complex mixture.¹³⁹ The racemate (XLIII) is hydrolyzed with hydrochloric acid to remove the acetyl group, and the product is resolved into its optical antipodes by crystallization of its salt with an optically active acid such as tartaric acid or *D*-camphorsulfonic acid.¹³⁸ The *D*-threo salt of the latter acid forms the least soluble component of the mixture. After separation, the salt is regenerated into the base (XLIV) by adding ammonium hydroxide. The final step in the synthesis is achieved by reacting XLIV with methyl dichloroacetate at elevated temperatures to yield XXXIII, the antibiotic identical with natural chloramphenicol.^{133,139}

A great number of analogs and structurally related compounds of chloramphenicol have been prepared and studied for their antibacterial properties. The nitro group in the molecule has been replaced by a large variety of substituents, and the dichloroacetamido group has been extensively modified. Moreover, many compounds which differ from the antibiotic in the structure of the side chain¹⁴⁹ have been synthesized. Much of this work indicates that changes in the aliphatic portion of the antibiotic molecule bring about substantial reduction or complete loss of antibacterial, antirickettsial, or antiviral activities. An analog, α -dichloroacetamido- β -hydroxy-*p*-nitropropionophenone,¹⁵⁰ in which the hydroxyl group on C-1 of the antibiotic was replaced by a keto group, was

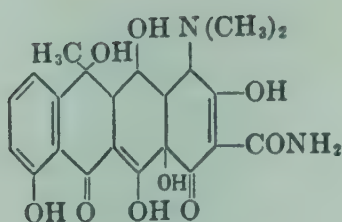
found to have slight activity against *Candida albicans*. In contrast to the changes which are produced by alterations in the aliphatic portion of the antibiotic molecule, modification in the aromatic ring does not produce such drastic results; for example, the *p*-methylsulfonyl analog¹⁵¹ has a high order of biologic activity. Marked antibacterial activity has been demonstrated in certain compounds in which the *p*-nitrophenyl group has been replaced by other aryl groups. The aromatic character of the ring structure, however, appears essential for the biologic activity.⁴⁷⁷ Activity resides in *D*-threo-1-biphenyl-2-dichloroacetamido-1,3-propanediol, in the corresponding racemic *p*-methylbiphenyl, and *p*-bromobiphenyl,¹⁴⁸ and in certain 1-biaryl derivatives.¹⁵² A novel series of 1-aza-3,7-dioxabicyclo [3.3.0] octanes derived from the chloramphenicol skeleton was prepared by Edgerton, Fisher, and Moersch,⁴⁷⁸ but their activities, if any, were not reported.

Chloramphenicol has an intensely bitter taste, and for this reason attempts have been aimed at the preparation of more palatable forms.⁵⁰⁷ A tasteless dosage form has been provided by the esterification of the primary hydroxyl group in chloramphenicol with palmitic acid.^{153,154} The chloramphenicol palmitate is inactive *in vitro* but is slowly hydrolyzed enzymatically in the duodenum with release and absorption of chloramphenicol from the intestinal tract. More than 90% of the antibiotic excreted in the urine is in the form of a water-soluble conjugate with glucuronic acid. The glucuronide-antibiotic conjugate can be hydrolyzed enzymatically by β -glucuronidase or alkali to regenerate free chloramphenicol.¹⁵⁵

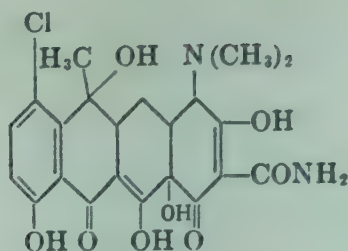
(E) THE TETRACYCLINE GROUP

The tetracycline family of antibiotics—oxytetracycline (XLV),¹⁵⁶ chlortetracycline (XLVI),¹⁵⁷ bromtetracycline,⁴⁸² 6-demethyltetracycline (XLVIa),⁴⁷⁹ 7-chlor-6-demethyltetracycline,⁴⁷⁹ and tetracycline (XLVII)^{158,159,160,161}—are crystalline, broad-spectrum antibiotics produced by certain species of *Streptomyces*.^{156,157,161} Tetracycline can also be prepared by the hydrogenolysis of chlortetracycline.^{159,160} These antibiotics are of great therapeutic value, being characterized by their wide antimicrobial activity against gram-positive and gram-negative bacteria, Rickettsiae and the psittacosis-lymphogranuloma viruses.^{162,163,164,165,166,167,168,169,170,171} In addition, the antibiotics are of considerable commercial importance because of their low toxicities.¹⁷² Chlortetracycline was the first of this group of antibiotics to be discovered.^{157,196}

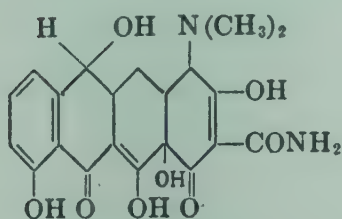
Oxytetracycline, chlortetracycline, and tetracycline are soluble in glycol ethers, pyridine and dilute acid and alkali; they are very slightly soluble in water and in lower molecular weight alcohols, and insoluble in



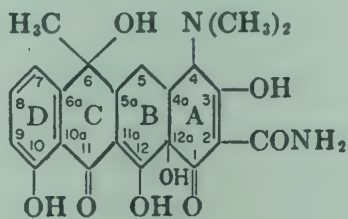
Oxytetracycline (XLV)



Chlortetracycline (XLVI)



6-Demethyltetracycline (XLVIa)



Tetracycline (XLVII)

ether and hydrocarbons. These antibiotics are amphoteric and readily form salts with strong acids and bases.^{173,174} The acid salts are well-formed crystalline compounds with high solubilities in water, but unless excess acid is present the crystalline amphoteric antibiotics separate on standing.¹⁷⁵ Tetracycline and oxytetracycline form salts with potassium or sodium hydroxide. All salts of this group of antibiotics darken slightly when exposed to strong direct sunlight.^{174,176} The tetracycline antibiotics form stable chelates with many metals. Various interesting complex salts with calcium, magnesium, aluminum, iron, and others have been prepared and studied for their chemical and antimicrobial properties. Albert⁴⁹⁰ has suggested that the antibacterial action of these antibiotics depends upon their metal binding properties. However, certain degradation products of tetracycline exhibit chelating properties, but no biologic activity.⁴⁹¹

These antibiotics contain a common skeleton which may be regarded as an octahydro analog of the hydrocarbon naphthacene (XLVIII).



Naphthacene (XLVIII)

Disclosure of the ring system depends upon a vital sequence of reactions. Reduction of the antibiotics with zinc and acetic acid successively removes the dimethylamino group and the oxygen at C-12a. The final reduction

product loses one molecule of water on treatment with acids, giving an insoluble anhydro-compound. When this substance is distilled from zinc dust, naphthacene (XLVIII) is obtained, thus establishing a linear tetracyclic structure for this series of antibiotics^{158,177,178} (Scheme 12).

Tetracycline^{158,159,160} is structurally related to chlortetracycline^{158,178} and oxytetracycline.¹⁷⁷ Tetracycline (XLVII) is 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. Chlortetracycline (XLVI) is the 7-chloro, and oxytetracycline (XLV) is the 5-hydroxy analog of tetracycline. Despite the close structural similarities of the antibiotics, they differ in their stability and solubility properties, infrared spectra, paper chromatographic patterns, in distinct colors developed with concentrated sulfuric acid, and also by their variation in the sensitivity of strains of the same species of microorganisms. The removal of chlorine from chlortetracycline stabilizes the molecule to destruction by bases. In addition, the antibiotics of this group possess certain other distinguishing chemical features since each undergoes a series of characteristic degradation reactions. Interpretations of spectrographic data of acid and base hydrolytic reactions were particularly helpful in providing information that led eventually to the elucidation of the total structure of these complex molecules.

In neutral solutions tetracycline, oxytetracycline, and chlortetracycline exist largely as zwitterions. The hydrochloride salts exhibit three acidity constants ranging from pK_a 3.3 to 9.7.^{177,178} It has been difficult to relate the pK_a values to specific functions. Inspection of formulas XLV, XLVI, XLVII will show that these substances contain three acid groupings of different types. The general formula written as an acid salt (XLIX) can be sectioned to show three distinct acid groups: the tricarbonylmethane system A, the ammonium cation B, and the phenolic

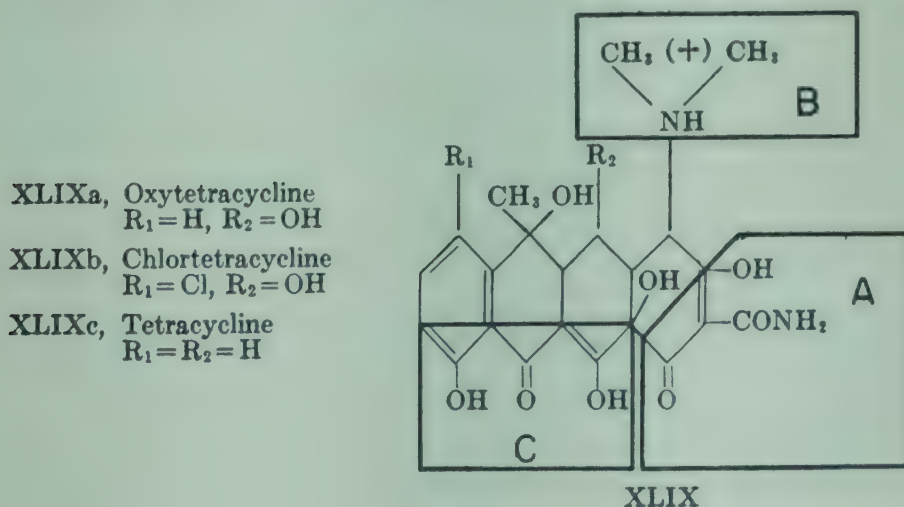


TABLE 2-2

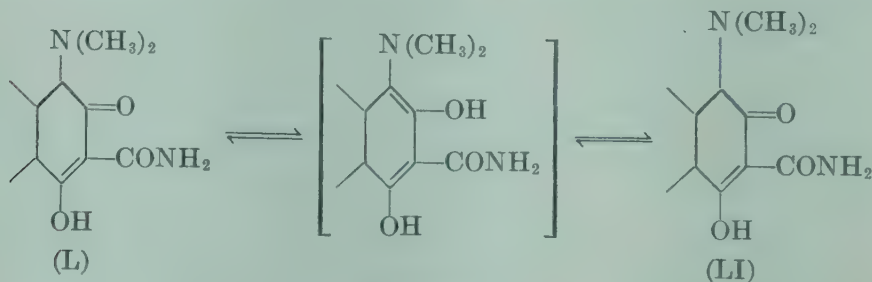
pK_a VALUES (OF HYDROCHLORIDES) IN AQUEOUS SOLUTIONS AT 25°

	Systems: A B C		
Oxytetracycline (XLIXa)	3.27	7.32	9.11
Chlortetracycline (XLIXb)	3.30	7.44	9.27
Tetracycline (XLIXc)	3.30	7.68	9.69

diketone system C. Through several separate arguments Stephens, Murai, Brunings, and Woodward¹⁷⁹ have assigned the carefully corrected thermodynamic pK_a values, indicated in Table 2-2, for oxytetracycline, tetracycline and chlortetracycline.

For each of the members of the tetracycline family, a set of conditions has been found which catalyze the formation of an equilibrium mixture of two components. In the case of tetracycline itself, a 15% solution in water-methanol mixture containing 1 molar sodium dihydrogen phosphate produces, within 25 hours, an equilibrium mixture measured spectrophotometrically and microbiologically to be about a 1.5:1 mixture of tetracycline and its new isomer, designated "quatrimecin" by Doerschuk, Bitler, and McCormick¹⁸⁰ and "epitetracycline" by Stephens, Conover, Gordon, Pennington, Wagner, Brunings, and Pilgrim.¹⁸¹ The new epimer was isolated as a crystalline, homogeneous ammonium salt. Similar epimeric forms have been prepared from oxytetracycline, chlortetracycline, bromtetracycline¹⁸² and the 6-demethyltetracyclines.⁴⁷⁹ For each of the five epimeric pairs, the equilibrium mixture is composed of approximately equal mixtures of the particular tetracycline and the 4-*epi*-tetracycline. The *epitetracyclines* have been isolated from the equilibrium mixtures, crystallized, and characterized.^{195,488} The new isomers are all of lowered *in vitro* antibiotic activities; however, each still possesses broad *in vivo* antibiotic activity.^{180,479}

From studies designed to explain the chemical nature of the reversible isomerization of these antibiotics,^{181,183,195} there is ample evidence that racemization at C-4 of XLVII leads to the equilibrium depicted in the partial formulas L and LI (Scheme 8). This explanation is strongly sup-



Scheme 8

ported by a study of dedimethylaminotetracycline, a substance in which the dimethylamino group in tetracycline is replaced by hydrogen. This compound shows no change under the conditions which epimerize either tetracycline or *epitetracycline*.¹⁸¹ Further work to establish epimerization at C-4 took the form of eliminating the asymmetry at C-4 by reductive removal⁵⁴⁹ of the 4-dimethylamino group from the methiodides⁵⁴⁹ of tetracycline and quatrimecin.¹⁸³ The two methiodides were isomeric, distinguishable and reversibly interconvertible. After reductive removal of the trimethylammonium group from both methiodides,⁵⁴⁹ the two dedimethylamino substances were identical.¹⁸³

1. OXYTETRACYCLINE

Notwithstanding the earlier discovery of chlortetracycline, determination of the structure of oxytetracycline by Hochstein, Stephens, Conover, Regna, Pasternack, Gordon, Pilgrim, Brunings, and Woodward^{184,177} spearheaded the explicit relationship of the tetracyclines. These structural determinations revealed the antibiotic to be a new type of chemical structure (XLV). This work, as delineated in one general paper,¹⁷⁷ formed the basis for a structure determination of chlortetracycline^{158,178} and tetracycline.^{159,160}

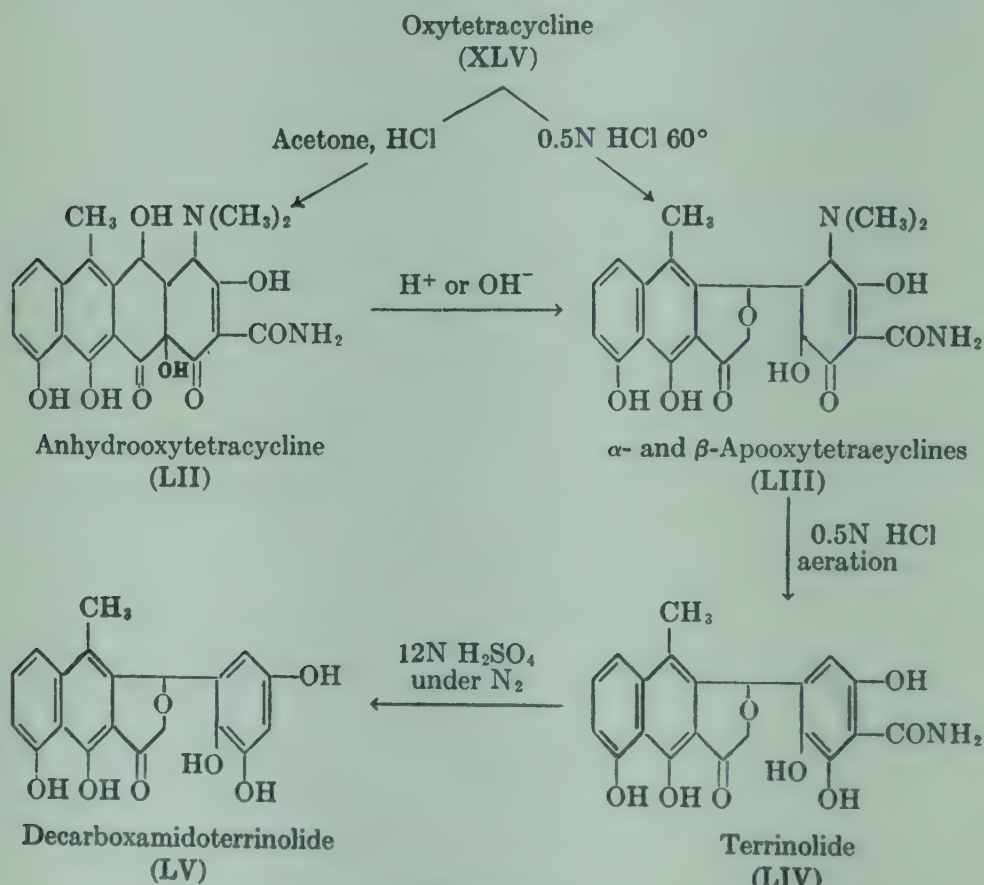
Oxytetracycline is a pale yellow, crystalline antibiotic produced by a species of actinomycetes, *Streptomyces rimosus*. The microorganism was isolated from a soil sample as a result of a planned research program and was so named because of the cracked appearance of its growth on the surface of an agar medium.¹⁵⁶ The antibiotic was isolated and characterized by Finlay, Hobby, P'an, Regna, Routien, Seeley, Shull, Sobin, Solomons, Vinson and Kane,¹⁵⁶ a group of workers which included specialists in many scientific disciplines other than chemistry.

The amphoteric antibiotic crystallizes as a dihydrate m.p. 181–182° C; $[\alpha]_D^{25} + 26.5^\circ$ (c, 1% in methyl alcohol); $[\alpha]_D^{25} - 196.6^\circ$ (c, 1% in 0.1 N hydrochloric acid); $[\alpha]_D^{25} - 2.1^\circ$ (c, 1% in 0.1 N sodium hydroxide).¹⁷⁵ It is commercially available as both the amphoteric base and the hydrochloride salt. The hydrochloride, m.p. 190–194° C, is a yellow crystalline substance and has a bitter taste. In addition, the antibiotic forms crystalline yellow-colored disodium and dipotassium salts¹⁷⁵ which are readily soluble in water and insoluble in alcohol. Oxytetracycline easily forms mixed salts, very insoluble in water, with a number of pairs of bivalent metal ions such as barium-calcium and barium-magnesium.¹⁷³ Further crystalline complexes of the antibiotic with calcium chloride,¹⁷³ magnesium chloride, etc. are readily produced. The unique polycarbonyl system of oxytetracycline prompts the ready formation of chelate

complexes with metallic ions. Multiple sites of metal binding are rendered possible by forces associated with hydroxyl and carboxamido functions. Conover, however, has presented evidence for the complex-forming center as the 11:12- β -dicarbonyl system of XLV.¹⁸⁷

The infrared spectrum of oxytetracycline shows no bands below 5.9μ and thus excludes the presence within the molecule of simple unconjugated aldehyde, ketone, carboxylic acid, ester and lactone functions. Certain other functional groups in the molecule, however, were readily established. A dimethyl derivative of the antibiotic fixed the presence of two phenolic or enolic functions, a diacetyl derivative identified two hydroxyl groups, a C-methyl group was detected by analysis, a dimethyl-amino group was liberated by alkaline hydrolysis and a carboxamide group was revealed by conversion of the amide to nitrile.¹⁷⁷ Further, the action of aqueous alkali liberated one mole each of ammonia and diethylamine, and from potassium hydroxide fusion, salicylic, m-hydroxybenzoic and succinic acids were isolated.^{185,186}

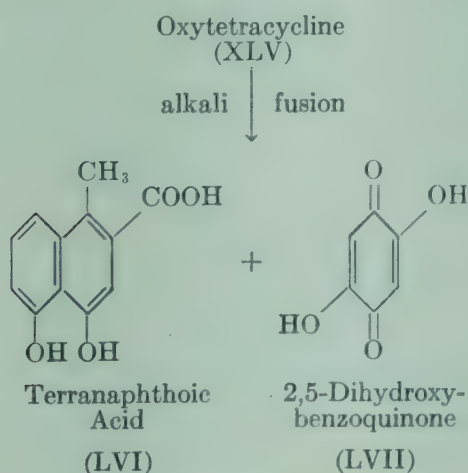
In contrast to the extensive fragmentation which takes place in alkaline media,¹⁸⁶ oxytetracycline undergoes stepwise degradation (Scheme 9)



Scheme 9

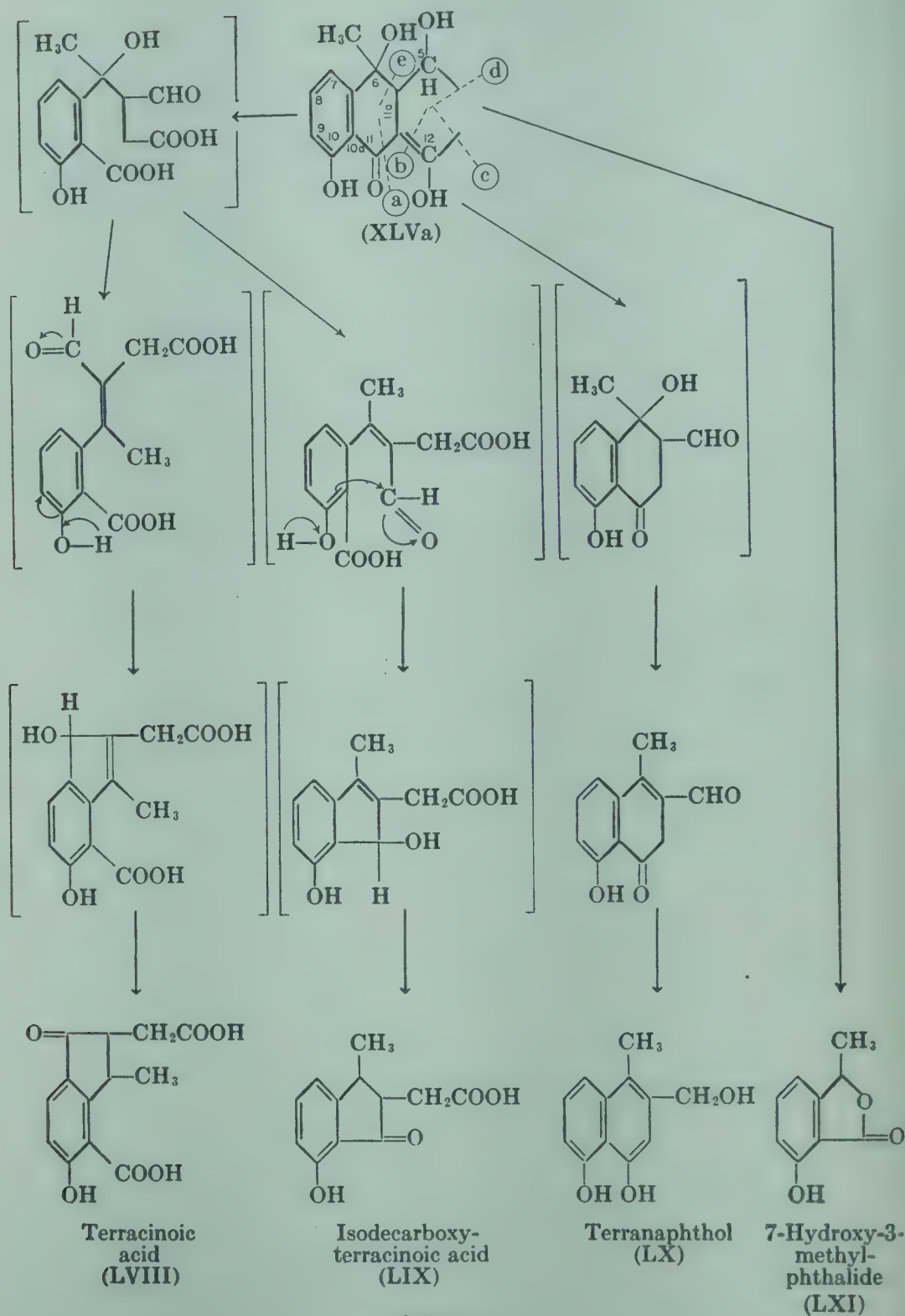
with successively stronger acids. Each product is related simply to its precursor. Since the C-12 hydroxyl hydrogen of oxytetracycline (XLV) is capable of enolization with the carbonyl group at C-11, and the tertiary hydroxyl group at C-6 situated α to the aromatic ring is subject to acid-catalyzed dehydration, all the acid degradation products possess the naphthalene system; and all but anhydrooxytetracycline (LII) contain it as the 8,9-dihydroxybenzophthalide system. Thus, the action of acidic reagents on oxytetracycline in nonaqueous media transforms it readily into the anhydrooxytetracycline (LII) which, like anhydrochlorotetracycline (LXVI), exhibits slight biologic activity.^{188,189,501} The substance LII is isomerized in dilute aqueous acids or more rapidly in dilute aqueous bases to a mixture of two stereoisomers α - and β -apooxytetracyclines, which exhibit characteristic lactone bands at 5.75μ in their infrared spectra.¹⁷⁷ These substances were the first transformation products isolated from mild acid hydrolysis of oxytetracycline.¹⁹⁰ The apooxytetracyclines are converted into terrinolide (LIV) by the loss of the dimethylamino group at 60°C in 0.5 N hydrochloric acid containing a trace of air or oxidizing agent.¹⁹¹ Treatment of LIV with boiling 12N-sulfuric acid causes the loss of ammonia and carbon dioxide, and yields the optically-inactive decarboxamidoterrinolide (LV).¹⁹¹

The presence of the 1,8-dihydroxybenzophthalide system in the acid degradation products received further confirmation from alkali fusion of the apooxytetracyclines. From the fusion products (Scheme 10), ter-



Scheme 10

ranaphthoic acid (LVI) was isolated, as well as a most significant substance shown to be 2,5-dihydroxybenzoquinone (LVII). The latter product gave support to previous assumptions that a six-membered car-

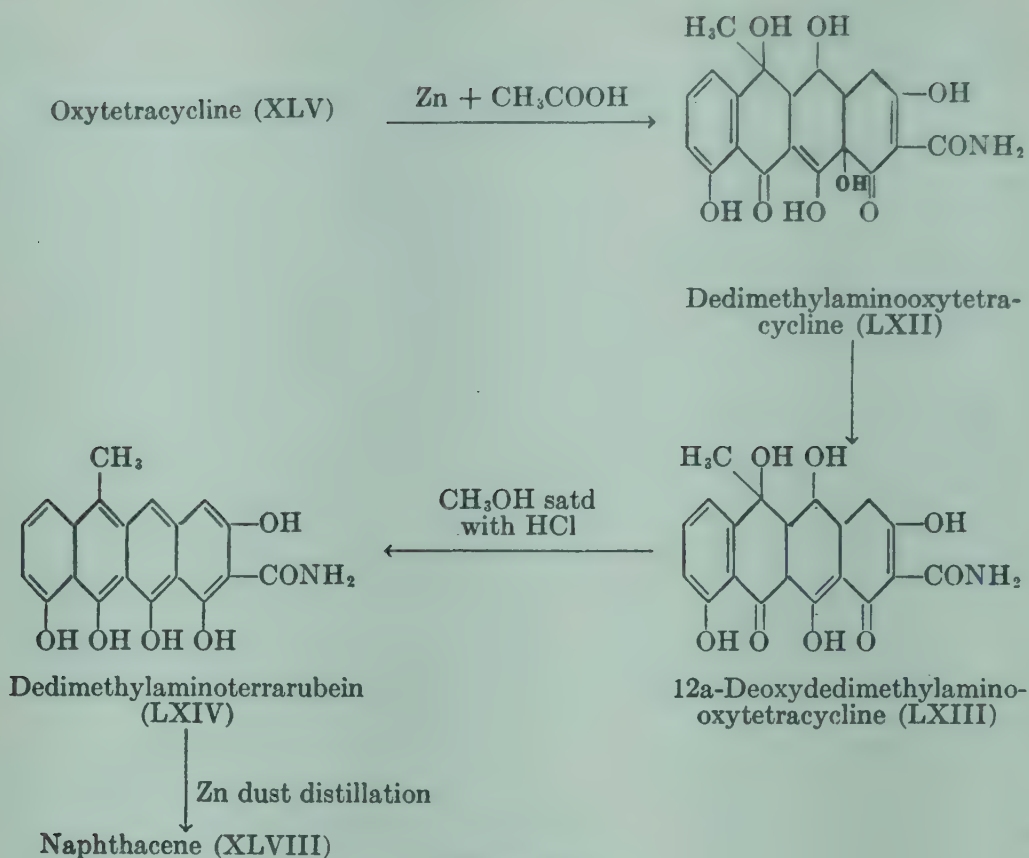


Scheme 11

bicyclic ring was attached to the benzophthalide unit.¹⁷⁷ The structure of LVI was established by synthesis.⁴⁸¹

The major product of the action of aqueous alkali on oxytetracycline is terracinoic acid (LVIII) which can be isolated as a well-defined crystalline substance.¹⁹² However, the degradation product was found to be racemic. This fact offered the first clue that the five-membered ring was not present as such in the oxytetracycline molecule, but was formed by a secondary ring closure during degradation.¹⁷⁷ Further interpretation of the alkaline reaction was gained from the successful search for small amounts of other predicted fragments which arise when oxytetracycline is hydrolyzed with hot aqueous alkali in the presence of zinc, and also from the synthesis of a series of model indanone compounds.¹⁹³ Several degradation products are isolated which are the end products of alternative modes of decomposition of the same portion of the oxytetracycline molecule.^{192,177,194} The reaction sequence shown in Scheme 11 would allow for the fragments isolated under these conditions. If bonds *a*, *c*, and *d* are cleaved, dehydration at C-6 results in an unstable, primary non-symmetric precursor and is followed by bond formation (C-5 → C-7) to give a new five-membered carbocyclic ring. In the presence of a base this compound is converted to a well-defined, racemic crystalline acid, terracinoic acid (4-carboxy-5-hydroxy-3-methylindanone-2-acetic acid, LVIII). An alternative cyclization (C-5 → C-10a) followed by decarboxylation, yields isodecarboxyterracinoic acid (7-hydroxy-3-methylindanone-2-acetic acid, LIX). Cleavage of bonds *b* and *d* liberates an aldehyde function at C-5. Loss of water at C-6 by a base-catalyzed elimination is followed by aromatization and reduction by zinc to form terranaphthol (3-hydroxymethyl-4-methyl-1,8-naphthalenediol) (LX). 7-Hydroxy-3-methylphthalide (LXI) is also found among the alkaline degradation products and results from cleavage at C-11 — C-11a and C-5 — C-6.

Reductive degradation of oxytetracycline also provides a distinct series of products leading to naphthacene (Scheme 12) which were of particular value in establishing the linear tetracyclic structure.¹⁷⁷ Mild reduction with zinc in acetic acid removes the dimethylamino group from oxytetracycline to give dedimethylaminooxytetracycline (LXII).⁵⁰² Prolonged action with the same reagents causes the loss of the C-12a oxygen and yields deoxydedimethylaminooxytetracycline (LXIII). Under mild acid conditions LXIII loses two molecules of water and forms a highly insoluble orange-red compound, dedimethylaminoterrarubein (LXIV), which contains a totally aromatic and conjugated system. Zinc-dust distillation of LXIV yields the aromatic hydrocarbon naphth-



Scheme 12

acene (XLVIII)¹⁷⁷ and thus provides the evidence for the basic skeleton of the antibiotic.

2. CHLORTETRACYCLINE

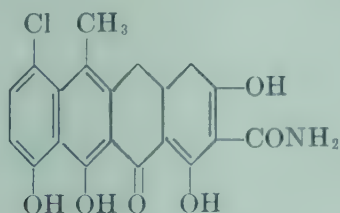
Chlortetracycline (XLVI) was isolated from *Streptomyces aureofaciens* by B. M. Duggar in 1948.^{157,196} Pure crystals of chlortetracycline are yellow-colored and melt at 172–174°. The antibiotic readily forms a crystalline hydrochloride: m.p. 234–236°; $[\alpha]_{25}^D - 235^\circ$ (water).¹⁷⁸ Bicarbonate solutions of the antibiotic are relatively unstable as compared with tetracycline. The approximate half-life of chlortetracycline is 1–3 hours, while that of the dechloro-derivative (XLVII) is about 24–30 hours. Chlortetracycline is slightly soluble in ordinary organic solvents, somewhat more soluble in water (0.55 mg. per ml. at 25°C), and insoluble in ethers.¹⁷⁴ More recently, *S. aureofaciens* mutants have been found which produce 7-bromotetracycline.^{182,482} Moreover, the halide metabolism of two classes of mutants was studied by Doerschuk and co-workers¹⁸² who showed that Class I contains mutants which utilize chloride ions for 7-chlortetracycline within limits which are independent of the concentra-

tion of the chloride ion. In contrast, the utilization of chloride ions by Class II mutants depends on the concentration of chloride.¹⁸²

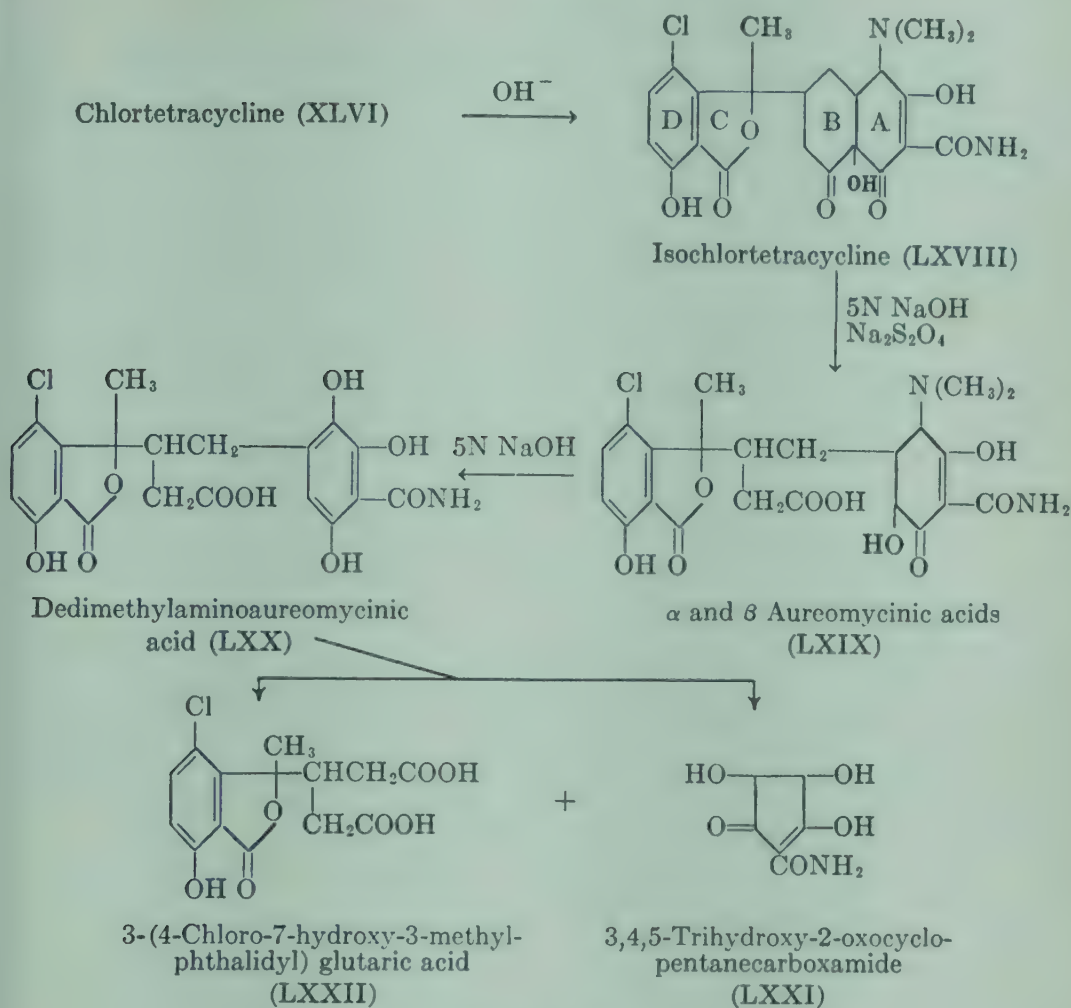
With the establishment of XLV as the structure of oxytetracycline,^{177,184} Stephens, Conover, Pasternack, Hochstein, Moreland, Regna, Pilgrim, Brunings, and Woodward¹⁷⁸ showed experimentally that chlortetracycline has structure XLVI.^{158,160,178} From a series of independent degradation studies^{197,198,199,200} Waller, Hutchings, Broschard, Goldman, Stein, Wolf, and Williams also suggested structure XLVI as one of two possibilities for chlortetracycline.²⁰¹

Erroneously interpreted X-ray studies purported to show that chlortetracycline and oxytetracycline differed only in the replacement, in the same position, of an atom of chlorine in chlortetracycline for an hydroxyl in oxytetracycline.^{202,203} However, this suggestion was untenable in the light of products obtained from alkaline fusion. Under these conditions chlortetracycline produced 5-chlorosalicylic acid, while salicylic acid was recovered from drastic treatment of oxytetracycline.^{186,204} The work on oxytetracycline and chlortetracycline had shown that the oxygen atoms at C-1, C-2, C-3, C-10, C-11, C-12 and C-12a were associated with acidity, characteristic ultraviolet absorption, and enolization within the molecule. Further, it was deduced that the remaining oxygen in chlortetracycline was linked as hydroxyl to position 6, since acid treatment of chlortetracycline, as with oxytetracycline, results in naphthalenoid absorption (Schemes 9 and 13). The placement of the chlorine atom at C-7 was further substantiated when chlortetracycline was hydrogenated over palladized carbon to yield tetracycline (XLVII), a dechloro product which exhibits ultraviolet absorption similar to that of oxytetracycline.^{159,160,178,205}

The hydronaphthacene skeleton common to this family of antibiotics was established in chlortetracycline by a series of reactions analogous to those shown in Scheme 12 for oxytetracycline. Mild zinc reduction of chlortetracycline yields dedimethylaminochlortetracycline, which on further vigorous reduction gives the insoluble anhydro compound (LXV) by loss of the C-12a oxygen atom. Zinc dust distillation of deoxydedimethylaminochlortetracycline (LXV) yields naphthacene (XLVIII).¹⁷⁸



Deoxydedimethylaminoanhydrochlortetracycline
(LXV)

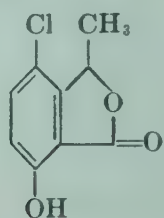


Scheme 14

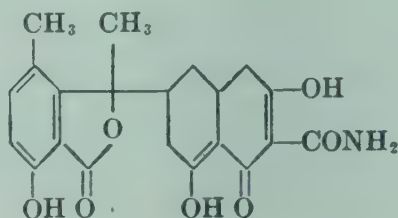
cycline (XLVI), is cleaved under conditions to give a carboxyl (acid) group which immediately lactonizes to give the phthalide, thus forming a new ring C in isochlortetracycline (LXVIII).¹⁹⁷ The action of somewhat stronger alkali containing sodium hydrosulfite on LXVIII forms α and β aureomycinic acids (LXIX) by a ketonic hydrolysis, and the production of a carboxyl group derived from the nonconjugated β -diketone of LXVIII.^{198,200,201,197,208,199} In 5 N alkali, LXIX is transformed into dedimethylaminoareomycinic acid (LXX) by the elimination of dimethylamine. Prolonged action of alkali and oxygen splits ring A from the molecule with subsequent contraction to a five-membered ring (LXXI).¹⁹⁷ Simultaneously, ring B develops the glutaric acid side chain attached to the phthalide of LXXII which originally was part of the isochlortetracycline molecule.^{178,208,199} The basic degradation products of chlortetracycline and oxytetracycline differ. In the case of oxytetracycline, the pres-

ence of the C-5 OH group permits more extensive aldole type reactions, thus yielding a wider variety of basic degradation products.

Conclusive evidence for the identity of the A/B ring system relationships of chlortetracycline and oxytetracycline was provided by subtracting the ultraviolet absorption spectrum of 4-chloro-7-hydroxy-3-methylphthalide (LXXIII) from that of isodeoxydedimethylaminochlortetracycline (LXXIV) which can be prepared from LXV by treatment with



4-Chloro-7-hydroxy-3-methylphthalide
(LXXIII)



Isodeoxydedimethylaminochlortetracycline
(LXXIV)

alcoholic potassium hydroxide. The difference curve offered indisputable evidence that the chromophoric system associated with rings A and B, and established in oxytetracycline, was present also in the chlortetracycline analog.¹⁷⁸ Thus, these collective arguments led to the assignment of chlortetracycline as 7-chlorotetracycline (XLVI).

3. TETRACYCLINE

Tetracycline (XLVII) lacks the 5-hydroxyl group of oxytetracycline and the 7-chloro group of chlortetracycline.¹⁵⁸ It was first prepared by Conover⁵¹² by selective catalytic hydrogenolysis of chlortetracycline. Further reports were published by Conover, Moreland, English, Stephens, and Pilgrim¹⁶⁰ and by Booth, Morton, Petisi, Wilkinson, and Williams.¹⁵⁹ Tetracycline has also been produced by Minieri, Firman, Mistretta, Abbey, Bricker, Rigler, and Sokol in fermentations of a *Streptomyces* species isolated from a sample of Texas soil¹⁶¹ and by other mutants of certain *Streptomyces*.¹⁸²

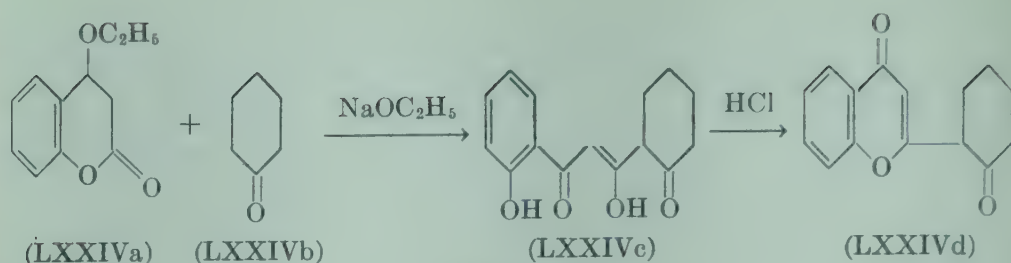
Tetracycline crystallizes from water-solvent mixtures as a trihydrate. The anhydrous form can be obtained by drying at 60° *in vacuo*; m.p. 170–175°C; $[\alpha]_D^{25} - 239^\circ$ (in methyl alcohol); solubility in water about 0.35 mg. per ml. at 25°C. Tetracycline hydrochloride is a yellow-colored crystalline salt which melts at about 214°C^{159,160}; $[\alpha]_D^{52} - 258^\circ$ (in 0.1 N hydrochloric acid); solubility in water 132 mg. per ml. at 26.5°C.

During the preparation of tetracycline^{158,159,160} from chlortetracycline in the presence of palladium catalyst, one mole of hydrogen is absorbed, and one mole of hydrochloric acid is formed by removal of the chlorine

substituent. No isomerization takes place during this reaction, since the characteristics of the ultraviolet absorption spectra of tetracycline in acidic and basic media are very similar to that of oxytetracycline.^{177,178} Confirmation of the structure of tetracycline has been obtained by dehydration of the antibiotic, either by methanolic hydrogen chloride^{160,201} or with hydrogen iodide to yield anhydrotetracycline (LXVII), the properties of which are closely related to those of the corresponding anhydrocompounds of oxytetracycline (LII) and chlortetracycline (LXVI). The substance LXVII is identical with the reaction product obtained by treatment of chlortetracycline with hydriodic acid (Scheme 13).^{178,201}

The stability of tetracycline is comparable to that of oxytetracycline, and is in contrast to chlortetracycline which is substantially less stable in basic solution.^{174,201} However, alkaline degradation of tetracycline follows the course adduced for chlortetracycline, since it lacks the C-5 hydroxyl group.²⁰⁸ Under more drastic alkaline conditions, tetracycline can be converted to isotetracycline, analogous to LXVIII but lacking the chlorine atom in the D-ring (Scheme 14). In the presence of oxygen, the A and B rings are cleaved with simultaneous elimination of the dimethylamino group, subsequent contraction of ring A to a cyclopentane derivative, and opening of the B-ring to yield a glutaric acid side chain on the phthalide,²¹⁰ similar to LXXII. The cyclopentane derivatives (LXXI) from both tetracycline and chlortetracycline²¹⁰ are identical. This series of reactions confirmed deductions made from ultraviolet absorption spectra and other physical data that only the replacement of the chlorine atom by a hydrogen atom is involved during the reduction of chlortetracycline to tetracycline.

André and Ullberg⁴⁸³ produced radioactive tetracycline by the reduction of chlortetracycline with tritium, using platinum oxide catalyst in dioxan. The synthesis of radioactively labeled antibiotics increases greatly the possibilities for investigations of the pharmacology and mode of action of these substances.^{209,506} The multiple centers of optical symmetry in the tetracycline molecules present formidable problems to approaches based on chemical syntheses of these antibiotics. However, several investigations are underway to synthesize portions of the tetracycline structures.^{562,563} A series of benzophenone and biphenylmethane derivatives of oxytetracycline analogs have been prepared using the Baeyer, Friedel-Crafts, and Fries reactions.⁴⁸⁴ Smissman and Gabbard⁴⁸⁵ have attempted to prepare models of compounds containing the same four oxygen functions present in position 1, 10, 11, and 12 of the tetracycline molecule. Through a series of reactions shown in Scheme 14a, about a 6% yield of the interesting product LXXIVc was obtained. The enolic hydroxycoumarin was protected as the enol ether (LXXIVa) and reacted



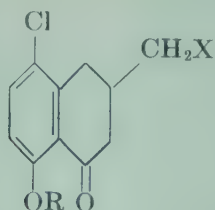
Scheme 14a

in sodium ethoxide with cyclohexanone (LXXIVb) to give 1-(2-hydroxyphenyl)-1-ethoxy-3-keto-3-(2-ketocyclohexyl)-1-propene. On acidification, this product yielded 1-(2-hydroxyphenyl)-3-(2-ketocyclohexyl)-propane-1,3-dione (LXXIVc) which in 10% aqueous hydrochloric acid was transformed into 2-(2-ketocyclohexyl) flavone (LXXIVd).⁴⁸⁵

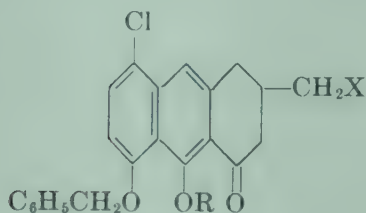
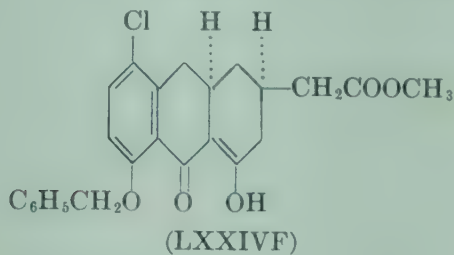
Boothe, Kende, Fields, and Wilkinson⁵⁶⁴ have succeeded with the total synthesis of the biologically active (\pm)-dedimethylamino-12a-deoxy-6-demethylanhydrochlortetracycline (LXXIVK). Its chemical identity has been established by comparison of characteristic infrared and ultraviolet spectra, bioassays, and chromatographic properties with a sample prepared from the natural antibiotic 6-demethylchlortetracycline (XLVIA).⁴⁷⁹

The tetraloneacetic acid (LXXIVA) was prepared by a series of reactions (Scheme 14b) in which diethyl sodiomalonate was alkylated with 2-chloro-5-methoxybenzyl bromide. The resulting benzylmalonic diethyl ester was reduced to the 1,3-diol with lithium aluminum hydride. The diol was reacted with bis-methanesulfonate, converted to the dinitrile, and hydrolyzed with alkali to yield the substituted benzylglutaric acid. Subsequent ring closure with polyphosphoric acid gave LXXIVA. After converting to the acid chloride (LXXIVB), Rosenmund reduction produced the corresponding aldehyde (LXXIVC), which was subjected to piperidine-catalyzed condensation with excess cyanoacetamide to a dicyanodiamide. Acid hydrolysis of the dicyanodiamide produced the demethyl phenolic diacid (LXXIVD). Reaction with benzyl chloride followed by esterification gave the diester (LXXIVE), which was cyclized by sodium hydride to the tricyclic ester (LXXIVF), tentatively assigned relative stereochemistry on conformational grounds.

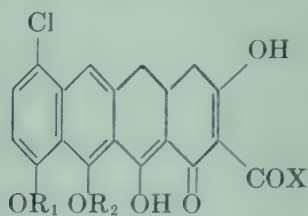
Angular bromination followed by dehydrobromination converted LXXIVF to the phenol (LXXIVG) which was transformed into the methyl ether and thence hydrolyzed to the free tricyclic acid (LXXIVH). Linear tetracyclic structure was effected by forming the acylmalonate LXXIVI, whereupon this compound was cyclized by sodium hydride to the ester (LXXIVJ). Fusion of LXXIVJ with ammonium formate, followed by dealkylation by hydrochloric acid in acetic acid gave (\pm)-amide



- LXXIVA $R = CH_3; X = COOH$
 LXXIVB $R = CH_3; X = COCl$
 LXXIVC $R = CH_3; X = CHO$
 LXXIVD $R = H; X = CH(CH_2COOH)_2$
 LXXIVE $R = CH_2C_6H_5; X = CH(CH_2COOCH_3)_2$



- LXXIVG $R = H; X = COOCH_3$
 LXXIVH $R = CH_3; X = COOH$
 LXXIVI $R = CH_3; X = COCH(COOC_2H_5)_2$



- LXXIVJ $R = CH_2C_6H_5;$
 $R_2 = CH_3; X = OC_2H_5$
 LXXIVK $R_1 = R_2 = H; X = NH_2$

Scheme 14b

(LXXIVK).⁵⁶⁴ The synthesis of this complex molecule furnishes particular support for the deduced structures of the tetracyclines.

4. BROMTETRACYCLINE

Sensi, de Ferrari, Gallo, and Rolland⁴⁸² working in Milano, Italy, isolated 7-bromotetracycline¹⁸² from fermentation broths of chlortetracycline-

producing streptomycetes grown on a medium containing sodium bromide. The antibiotic possesses antibacterial properties,⁴⁸⁶ and an ultraviolet absorption spectrum very similar to 7-chlortetracycline.⁴⁸² Crystalline bromtetracycline, m.p. 170–172°, forms a crystalline hydrochloride (dec. p. 235–240°) which shows $[\alpha]_D^{25} - 196^\circ$ in 0.1 N hydrochloric acid. In acid solution, bromtetracycline undergoes dehydration to form anhydrobromtetracycline⁴⁸² as shown in Scheme 13 for anhydrochlortetracycline. Similarly, in alkaline solution it forms isobromtetracycline⁴⁸² (see Scheme 14). Catalytic hydrogenolysis using palladium gives tetracycline⁴⁸² identical with tetracycline obtained by hydrogenolysis of chlortetracycline.¹⁶⁰

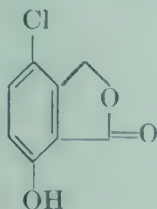
Doerschuk and co-workers¹⁸² have shown that a mutant of *S. aureofaciens* in the absence of chloride ion utilizes bromide for 7-bromtetracycline at a rate independent of bromide concentration over a specified range.

5. DEMETHYLTETRACYCLINE

McCormick, Sjolander, Hirsch, Jensen, and Doerschuk⁴⁷⁹ found that mutants of *S. aureofaciens* produced two antibiotics 7-chloro-6-demethyltetracycline ($[\alpha]_D^{25} - 258^\circ$; m.p. dec. 174–178°), and 6-demethyltetracycline (XLVIa) ($[\alpha]_D^{25} - 259^\circ$; m.p. dec. 203–209°), a new family of tetracycline antibiotics. The ultraviolet absorption spectra of these substances are virtually the same as those of their 6-methyl analogs. Like the latter substances, the demethyl antibiotics form *epi* isomers under epimerizing conditions to 7-chloro-6-demethyl-4-*epi*-tetracycline ($[\alpha]_D^{25} - 323^\circ$; m.p. dec. 214–216°) and 6-demethyl-4-*epi*-tetracycline ($[\alpha]_D^{25} - 335^\circ$; m.p. dec. 225–230°). In a preliminary experiment 7-chloro-6-demethyltetracycline was distilled with a highly active zinc to give naphthacene (XLVIII). Numerous C-methyl determinations of these antibiotics gave zero values.⁴⁸⁰

The great resistance of these compounds to degradation by acid and alkali was interpreted to mean that ring C was involved in the stabilization of the molecule. This stability is in contrast to the acid and alkali sensitivity of ring C in other tetracyclines.^{177,178,182,200} However, when 7-chloro-6-demethyltetracycline was heated in concentrated hydrochloric acid, ring C was aromatized giving an anhydro compound $[\alpha]_D^{25} + 105^\circ$; m.p. 205–210°. The formation of the anhydro compound was good evidence for an hydroxyl group on C-6 and a hydrogen atom on C-5a. On oxidation of the demethyl antibiotic with oxygen in sodium hydroxide, as expected, there was isolated dimethylamine, LXXI, and β -(4-chloro-7-hydroxyphthalide-3)glutaric acid which is similar to, but lacks the methyl group of LXXII. Further degradation reactions which established the structures of rings A, B, and D, were patterned after those carried out

with chlortetracycline (XLVI).⁴⁸⁰ In addition, the arrangement of substituents in the ring D and in portions of ring C of 7-chloro-6-demethyl-tetracycline was determined by degradation of the demethyl antibiotic to 4-chloro-7-hydroxyphthalide (LXXIVe) which is analogous to 4-



4-Chloro-7-hydroxyphthalide (LXXIVe)

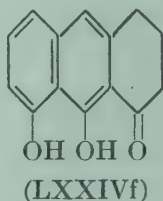
chloro-7-hydroxy-3-methylphthalide (LXXIII), obtained by the same route from chlortetracycline.¹⁷⁸ The structure of LXXIVe was established by synthesis.⁴⁸⁷

6. CHEMICAL STRUCTURE AND ANTIMICROBIAL ACTIVITY RELATIONSHIPS

It was realized early in these studies that constituents on C-5 and C-7 of the tetracycline molecule contributed little to the biologic activity.¹⁸⁹ Undiminished activity is characteristic also of the 6-demethyltetracycline,⁴⁷⁹ in which the C-6 methyl group is replaced by hydrogen. It might be expected that the dimethylamino function at C-4 is essential to the antimicrobial activity. However, Stephens and co-workers⁵⁰¹ have demonstrated that dedimethylaminooxytetracycline (LXII),¹⁷⁷ dedimethylaminotetracycline,¹⁸¹ and dedimethylaminochlortetracycline¹⁷⁸ show about 25% of the antibacterial effects of the respective parent antibiotics against substantially the same microbial spectra. In studying the relative activity of the dedimethylamino analogs with varying pH, higher activity is observed at pH 5.0 than at pH 7.0, indicating that the electrically neutral zwitterion is the most active ionic form. It is striking that stereochemical isomerizations of the dimethylamino group cause greater loss of activity than its complete removal. Since the C-4 *epi*tetracycline^{181,183,195,479} series exists in a conformation opposite to that of the parent antibiotics, Stephens⁵⁰¹ has suggested that specific conformational requirements must be fulfilled in order for the antibiotic molecule to orient itself properly to the vital enzyme surface.

5,12a-Diacetyloxytetracycline¹⁷⁷ appears to be devoid of *in vitro* antibacterial activity. Esterification of the aliphatic hydroxyl group at C-12a drastically reduces *in vitro* activity and may perhaps entirely curtail it. While the C-12a hydroxyl group appears to play an important role in activity, these studies strongly suggest that no simple moiety of

the tetracycline molecule can be considered the salient seat of biologic activity. Provided the transformation products fulfil other activity requirements—electronic, steric, or structural—certain nonessential functions can be altered without destroying activity.⁵⁰¹ On the other hand, the anhydrotetracyclines have a different activity mechanism than tetracycline types as exhibited by their very different antibacterial spectra. The key to their activity appears to reside in that part of the molecule corresponding to the three-ring model compound LXXIVf which exhibits appreciable activity.⁵⁰¹



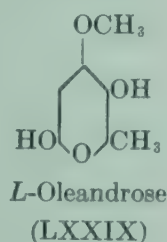
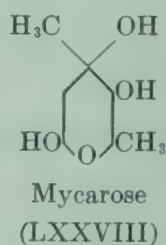
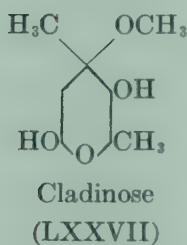
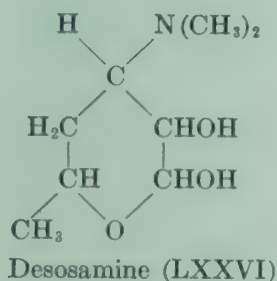
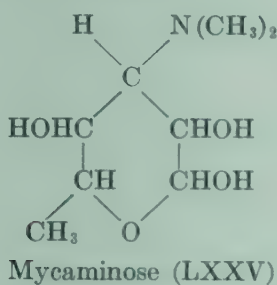
F. THE MACROLIDES

During the past several years the intensive interest in antimicrobial products has brought into existence a vast number of related basic antibiotics with moderately high molecular weights. Professor Robert B. Woodward has designated this class of substances “macrolides”, because they contain a macrocyclic lactone ring.²¹¹ Their exact composition has been subject to considerable uncertainty mainly because they readily form solvated crystals. The best defined members of this group and the year in which they were announced are: pikromycin (1951)^{212, 213, 489}; erythromycin (1952)²¹⁴; carbomycin (Magnamycin) (1952)²¹⁵; methymycin (1953)²¹⁶; spiramycin (1954)^{508, 509}; oleandomycin (1954)^{217, 218}; and narbomycin (1955)²¹⁹. The chemical structures of those which have been well-characterized suggest a biogenetic pattern constructed of simple units—for example, carbomycin appears to be composed chiefly of acetate units²¹¹ and erythromycin and methymycin of propionate polymers^{220, 221} which are more common among macrolide antibiotics.

This class of antibiotics exhibits similar patterns of antimicrobial activity. All are active principally against gram-positive bacteria²²² but are also effective against a few gram-negative bacteria and certain pathogens resistant to other common antibiotics. In general, the strains which produce each of these antibiotics synthesize, in minor amounts, a ‘B’ component, and in erythromycin even a ‘C’ component, with closely related chemical structures and antimicrobial patterns.^{218, 223, 224, 225, 472}

The macrolides embody unique nitrogen-containing sugars in their molecules. With the exception of carbomycin and spiramycin which contain a novel amino sugar called mycaminose (LXXV) (3-dimethyl-

amino-3-deoxy-5-methylpentopyranoside)^{226, 520} erythromycin,^{227, 228} pikromycin^{229, 361} oleandomycin,²¹⁸ methymycin,²³⁰ and narbomycin²¹⁹ all contain desosamine (LXXVI) (3-dimethylamino-4-deoxy-5-methylpentopyranoside). Desosamine is closely related to the amino sugar mycaminoside (LXXV) in carbomycin (LXXX). Erythromycin also contains a nitrogen-free carbohydrate, cladinose (LXXVII)^{227, 231, 232, 233}; carbomycin and spiramycin contain mycarose (LXXVIII)^{234, 518}; and oleandomycin, *L*-oleandrose (LXXIX).²¹⁸

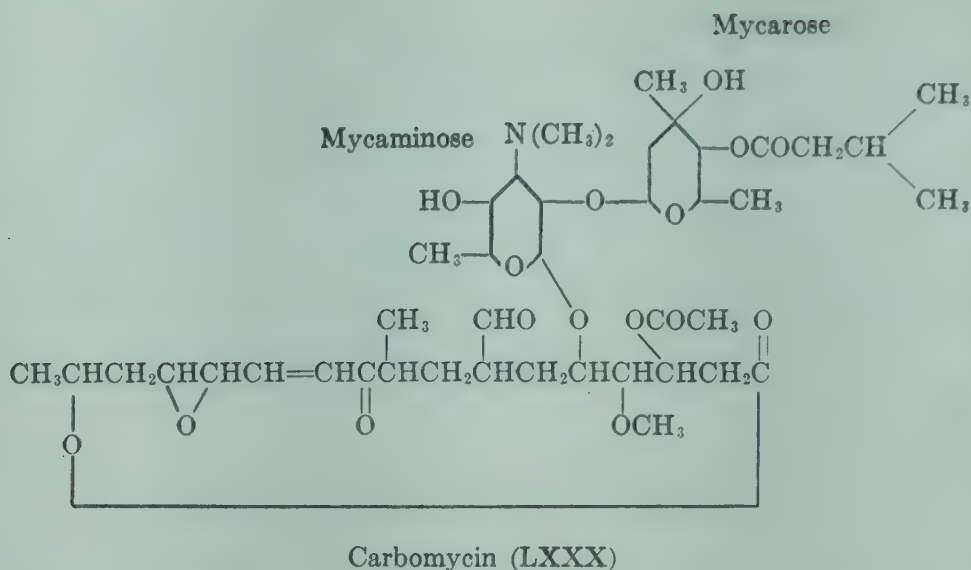


The structures of carbomycin, erythromycin, oleandomycin and methymycin are briefly described below; the first three are of clinical importance.

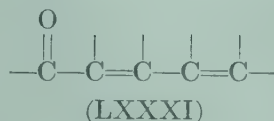
1. CARBOMYCIN

Carbomycin was isolated by Tanner, Lees, and Routein^{513, 215} and independently by Pagano, Weinstein, and McKee.²³⁵ The antibiotic is elaborated by strains of the microorganism *Streptomyces halstedii*,²¹⁶ which also produces a closely related B component.²²⁴ Both antibiotics are readily soluble in most organic solvents, and thus are readily extracted from filtered fermentation broth by water-immiscible solvents.

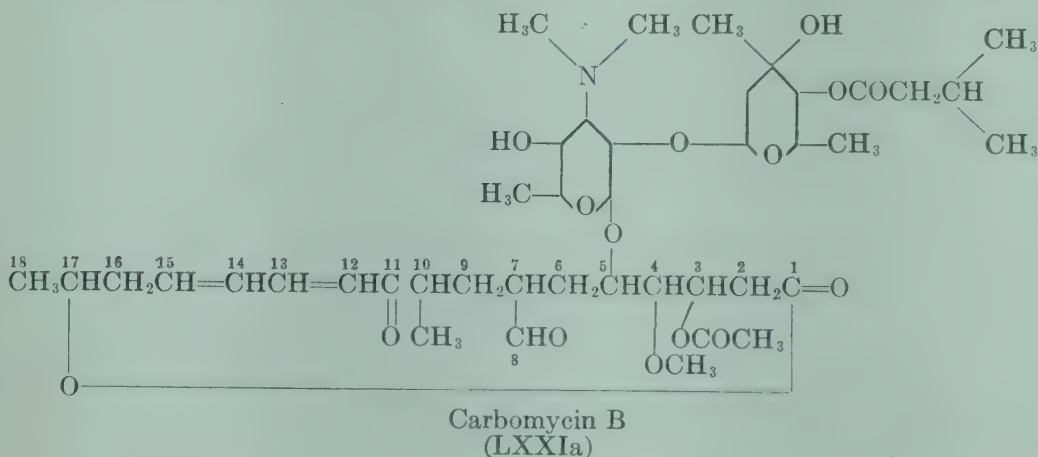
Carbomycin has the structure (LXXX) as shown by Woodward and his collaborators.²¹¹ The tentatively assigned empirical formula²³⁶ was established with certainty as C₄₂H₆₇NO₁₆ only after the complete structure had been determined.²¹¹ The antibiotic is a crystalline, monobasic substance²³⁷ pK_a 6.8–7.0²³⁶. It crystallizes well from methanol: m.p. 212–214°C., [α]_D²⁵ – 58.6° (in chloroform). The strong absorption peak in the ultraviolet at λ_{238mμ} and a weaker peak at λ_{327mμ} suggested the presence of an α,β-unsaturated carbonyl system.²³⁶



Another carbonyl function, not involved in this system, was inferred from the characteristic aldehyde band at 3.7μ in the infrared spectrum. The aldehyde group is involved in oxime, thiosemicarbazone, and dimethylacetal derivatives of the antibiotic. The intense absorption band of carbomycin disappears on catalytic hydrogenation of the carbon-carbon double bond.²³⁶ However, when carbomycin itself is reacted with potassium iodide in acetic acid, the product is found to contain the $\alpha,\beta,\gamma,\delta$ -doubly unsaturated carbonyl system (LXXXI). The crystalline sub-



stance which is derived from this reaction has the composition $\text{C}_{42}\text{H}_{67}\text{NO}_{15}$. It contains only one less oxygen atom than carbomycin and is identical with the natural B component found in culture filtrates along with carbo-



mycin. The differences between the two antibiotics reside in the replacement of the epoxide in carbomycin by a double bond in carbomycin B (LXXIa).²¹¹ In a highly commendable discussion of the biogenetic pattern of the macrolides, Woodward has proposed that carbomycin B is the precursor of carbomycin itself.²¹¹

The difficult elucidation of the complex structure of carbomycin depended not only on experimental evidence provided by fragmentation of the carbomycin molecule, but to a large extent on the B-component and both tetrahydro-derivatives. However, certain key reactions derived from carbomycin which were capable of being fitted into the structure design are indicated in Scheme 15.

Early degradation of carbomycin disclosed the presence of two unique sugars in the molecule. Regna, Hochstein, Wagner, Messina, Murai, and Woodward^{234,236} showed that methanolysis of the antibiotic in methanolic hydrochloric acid produced crystalline carimbose (LXXXII) with composition $C_{30}H_{47}NO_{42}$, and an oily neutral substance the methyl glycoside of 4-isovalerylmycarose (LXXXIII), which can be cleaved according to Scheme 15. The formulas are devoid of configurational implications. Alkaline hydrolysis of LXXXIII yields isovaleric acid (LXXXIV) and a mixture of anomeric methyl mycarosides (LXXXV). Aqueous acid hydrolysis of LXXXV yields mycarose (LXXVIII) as a crystalline solid.²³⁴ Vigorous acid hydrolysis of carimbose (LXXXII) provides an unusual amino sugar called mycaminose (LXXV).²²⁶ Oxidation of mycaminose with periodate salt yields one mole of formic acid per mole of amino sugar, and a new dimethylamino sugar $C_7H_{15}NO_3$ with one carbon atom less than mycaminose.²²⁶

Woodward and his collaborators at Harvard showed that carimbose has the cyclic skeleton indicated as LXXX, and further deduced the placement of the amino sugar, the oxygen functions and the presence of the unusual oxide system. The structure of the nucleus presented many difficulties, one of which was the almost intractable nature of intermediate degradation products of carimbose. However, several fragments were derived from this moiety. For example, in one series of step-wise degradations shown in Scheme 15, the placement of both carbonyl functions was established.

When carimbose was oxidized by excess periodic acid in the presence of potassium permanganate and the mixtures treated with hot aqueous alkali, the crystalline methoxy acid $C_{13}H_{18}O_7$ (LXXXVI) recovered was found to contain an $\alpha,\beta,\gamma,\delta$ -doubly unsaturated system. The action of mineral acid on LXXXVI transformed it into a new tribasic acid, $C_{12}H_{16}O_7$ (LXXXVII). On treatment of the keto-acid (LXXXVII) with alkali, the 2,3-double bond was cleaved and a C-10 keto-dicarboxylic acid

(LXXXVIII) was obtained. The acid (LXXXVIII) was found to be related to a C-9 acid which had been obtained from LXXXVII by the action of potassium permanganate and sodium periodate. It was evident, therefore, that the alkali treatment of LXXXVII had eliminated carbon atoms 1 and 2. Wolff-Kishner reduction of the keto-acid (LXXXVIII) produced a saturated acid (XC) which on vigorous treatment was converted to an alpha-substituted glutaric anhydride. In addition, ozonization followed by hydrogen peroxide treatment converted LXXXVI into the crystalline, saturated tribasic acid (LXXXIX). The same acid was obtained by hot nitric acid oxidation of LXXXVII. These facts required that the C-8 acid and ultimately the C-13 tricarboxylic acid possess the structures indicated in LXXXIX and LXXXVI, respectively. Moreover, the deduced structure of the C-8 acid was firmly established by synthesis, and thus reflected on the correctness of the structure of the C-13 acid.

Evidence for the lactone structure was obtained by a series of reactions which started with the tetrahydro derivatives of carbomycin or carimbose. In further step-wise reactions, platinum and acetic acid reduced the aldehyde group, and sodium borohydride reduced the ring carbonyl. Dimethylamine was liberated on vigorous alkali treatment of these fully saturated compounds. The product of this reaction showed a strong ultraviolet absorption at $265\text{ m}\mu$ that shifted to longer wave lengths when the solution was acidified. This behavior was interpreted as characteristic of an $\alpha,\beta,\gamma,\delta$ -unsaturated carboxylic acid system, evolved through the cleavage of a lactone ring. A further contribution toward the completion of the lactone picture was obtained by the nitric acid oxidation of tetrahydrocarbomycin-B, which yielded pimelic acid, demonstrating that one segment of the very long chain of carbon atoms contained five methylene groups.²¹¹

Woodward has pointed out that carbomycin contains seventeen asymmetric carbon atoms; thus the molecule can exist in no less than 262,144 diastereoisomers. However, studies carried out thus far have significantly reduced this gigantic number of possible formulations, so that the complete configurational structure may one day become known.

2. ERYTHROMYCIN

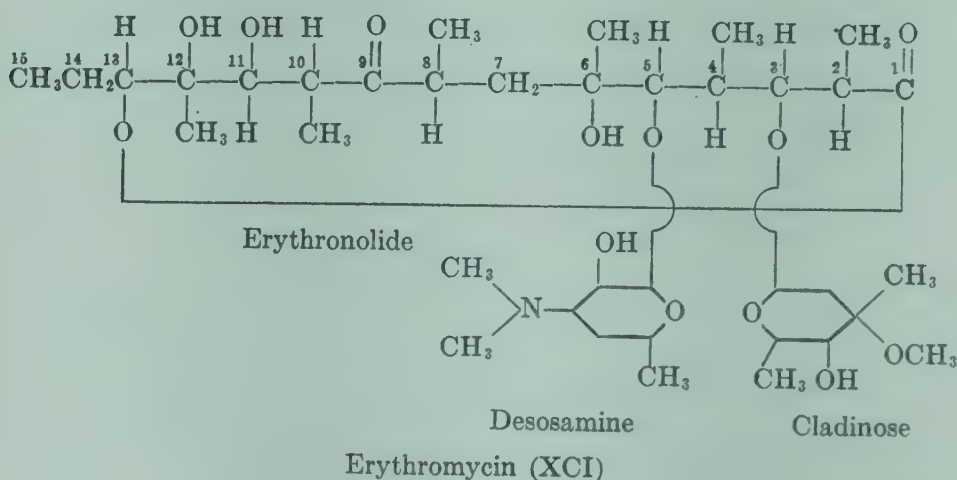
McGuire, Bunch, Andersen, Boaz, Flynn, Powell, and Smith²¹⁴ isolated erythromycin from culture filtrates of a strain of *Streptomyces erythreus* obtained from a sample of Philippine soil. A second crystalline antibiotic, erythromycin B, was reported by Pettinga, Start, and Van Abeele.²²³ Mother liquors, after removal of erythromycin and erythro-

mycin B, were found to contain a third component, erythromycin C.⁴⁷² The antibiotics have very similar properties. However, the B component has a greater acid stability than erythromycin²³⁸ and an antibiotic activity only about 75 to 85% as great.²²³ Erythromycin C has a comparable microbiologic spectrum.⁴⁷²

A metabolite of erythromycin has been isolated from the bile of dogs injected with erythromycin glucoheptonate. The desosamine (LXXVI) moiety of the antibiotic undergoes N-demethylation *in vivo*²³⁹ to de-N-methylerythromycin, a substance which is only about 5% as active as erythromycin. The metabolite can be converted to erythromycin by reductive methylation with formaldehyde and hydrogen.²⁴⁰ New erythromycins were synthesized from de-N-methylerythromycin by hydrogenation with the appropriate aldehyde, using palladium as catalyst. In this way, the ethyl-, propyl-, n-butyl-, isobutyl-, and γ -phenylpropyl-norerythromycins were prepared. None showed activity except the ethyl norerythromycin. However this derivative possesses only one-fifth the activity of erythromycin itself.⁴⁹³

Erythromycin is a weak basic crystalline substance, pK_a 8.6; m.p. 135–140°C; $[\alpha]_D^{25} - 73.5$ (in methanol). The hydrochloride, glucoheptonate, stearate, etc., are well-characterized crystalline salts. The antibiotic forms an interesting series of water-insoluble, substantially tasteless esters which, in general, hydrolyze to yield high microbiologic activity.²⁴¹ Erythromycin is soluble in organic solvents and only slightly soluble in water. In neutral solutions it is stable, but below pH 5 the drug loses its activity rather rapidly.²²⁷

Erythromycin has the composition $C_{37}H_{67}NO_{13}$ and structure (XCI). It has been shown to be a bisglycoside of a twenty-one carbon polyhydroxy ketolactone by Wiley, Gerzon, Flynn, Sigal, Weaver, Quarck, Chauvette, and Monahan.^{473, 220, 242} The presence of a ketolactone struc-



The placement of the ketonic carbonyl in erythromycin (XCI)⁴⁷³ has been established by degradation of erythralosamine (XCIIa) (Scheme 15a). Oxidation of XCIIa with chromic oxide gave XIIf, which on acid hydrolysis produced the crystalline neutral substance XCIIId, readily reducible to XCIIe. The saturated dilactone XCIIe showed the existence of two rings, no unsaturation, and the presence of four C-methyl groups. The strongest evidence for the structure of XCIIe was deduced from the diol XCIIIf, obtained by lithium aluminum hydride reduction of the dilactone (XCIIe), treatment of the product with sodium metaperiodate, followed by sodium borohydride reduction. The product XCIIIf was isolated as the bis-(3,5-dinitrobenzoate) of 2,4-dimethyl-1-5-pentanediol. Evidence for the δ -lactone portion of the dilactone XCIIe was obtained by isolation of the aldehyde acid (XCIIg) following alkaline hydrolysis of XCIIe and periodate oxidation. The γ -lactone portion of XCIIe was deduced from the isolation of α -methyllevulinic acid (XCIIh). Both carbonyl-containing compounds XCIIg and XCIIh were isolated as their dinitrophenylhydrazones. Structures of the degradation products were established by synthesis.⁴⁷³

An excellent argument is presented by Wiley and co-workers⁴⁷³ on the placement of the substituents on the aglycone portion of the erythromycin molecule. Further, several possible positions for cladinose are eliminated on the basis of a series of reactions which allow only for its placement on C-3. Desosamine was shown to be attached to a carbon atom adjacent to one joined to a free hydroxyl group.²⁴² There are only two such possibilities, C-11-C-12 and C-5-C-6. The conditions under which desosamine is eliminated require that it be placed on C-5. The assignment of the desosamine position is in agreement with similar deductions made on pikromycin⁴⁹² and methymycin.²²¹ Tentative assignment of the configuration of the asymmetric carbon atoms C-2, C-3, C-4, C-8, C-9, C-10, and C-13 in the erythromycin molecule (XCI) has been made by Gerzon and co-workers.²²⁰

a. Erythromycin B.

Erythromycin B has the molecular formula $C_{37}H_{67}NO_{12}$, and Wiley and co-workers⁴⁹⁴ have shown that it has structure CXIII. It differs from erythromycin in having one less oxygen atom.²³³ Like erythromycin it contains desosamine²²⁸ (LXXVI) and cladinose²⁴³ (LXXVII). Mild acid hydrolysis of erythromycin B yields cladinose and an amorphous compound which does not form a spiroketal as does erythromycin in the presence of acid.^{242,473}

Reduction of erythromycin B with sodium borohydride, followed by mild acid hydrolysis of the intermediate dihydroerythromycin B, yields

C₁₂-tetrol (XCIIk), plus the methyl ketone function in XCIIj, account for the 21 carbon atoms of the aglycone portion of XCIII. These data were supported by a series of reactions all of which were consistent with the structure of erythromycin B (XCIII).⁴⁹⁴

b. Erythromycin C

Erythromycin C was detected by paper chromatography in fermentation broths containing erythromycin and erythromycin B.⁴⁷² Wiley, Gale, Pettinga, and Gerzon⁴⁷² have shown that erythromycin C has the molecular formula C₃₆H₆₅NO₁₃. The methoxy group present in the neutral sugar cladinose of erythromycin (XCI) is absent in erythromycin C. Consequently it was subjected to the same degradative reactions used in the erythromycin series.⁴⁷³ Acid methanolysis of erythromycin C produced erythralosamine (XCIIa), identical with that isolated from erythromycin. Sodium borohydride reduction to dihydroerythromycin C, followed by acid methanolysis, formed 5-O-desoasminyldihydroerythronolide previously isolated from erythromycin.⁴⁷³ The isolation of these products, well-established from the degradation of erythromycin, suggests that erythromycin C differs only with respect to the methoxy group attached to the cladinose (LXXVII) moiety of erythromycin.⁴⁷³

3. OLEANDOMYCIN

Oleandomycin (PA 105), in itself a therapeutically useful member of this class of substances,²⁴⁶ has attracted further attention because of its synergistic action against certain microorganisms when in combination with oxytetracycline and tetracycline.^{246,247,248,249,250,251,252,254} Sobin, Routien, and Lees⁵¹⁴ found that the antibiotic is elaborated by a strain of *Streptomyces antibioticus* grown under submerged aerobic conditions.²⁵³ Certain other conditions of fermentation induce the formation of a closely related substance designated oleandomycin B. Oleandomycin can be extracted from culture filtrates by methyl isobutyl ketone. On addition of hydrochloric acid, the antibiotic can be crystallized from the methyl isobutyl ketone as the hydrated hydrochloride salt: m.p. 134–135°C.; $[\alpha]_D^{25} - 54^\circ$ (in methanol).²⁵³

Els, Murai, and Celmer^{218,505} have demonstrated the basic nature of the antibiotic, have assigned to it a formula of C₃₅H₆₁NO₁₂, and have suggested that its structure has many of the features of the macrolides. The purified antibiotic is very soluble in many organic solvents; m.p. 110° (dec.); pK_a 8.6; $[\alpha]_D - 65^\circ$ (in methanol). Oleandomycin forms solvated crystals, particularly with halogenated hydrocarbons, from which the bound solvent is removed only with difficulty.^{218,505}

Like other members of its class of substances, the antibiotic exhibits two sharply defined carbonyl bands in the infrared spectrum. A biologically inactive substance is obtained when the ketone function is reduced with sodium borohydride. An active "anhydro-oleandomycin" $C_{35}H_{59}NO_{11}$ is formed by a base-catalyzed conversion of the antibiotic, which is relatively resistant to alkaline hydrolysis; however, continued saponification opens the lactone ring.

Mild acid hydrolysis of oleandomycin gives a basic substance $C_{28}H_{51}NO_9$ and a neutral substance shown to be the pyranoside form of *L*-oleandrose (LXXIX), a substance also derived from the oleander plant.^{255,256} More vigorous hydrolysis of the basic fragment in acid media liberates desosamine hydrochloride (LXXVI), a characteristic component of a number of these antibiotics. The placement of these sugars as glycosidic substituents on a C-20 ring has not yet been deduced. However, the residual $C_{20}H_{36}O_7$ fragment is a lactone containing a ketone, at least three hydroxyl groups (two are sites of glycosidic linkages), and a minimum of six C-methyl substituents.²¹⁸ With the partial structure so well-advanced, the complete formulation of oleandomycin is fairly well assured.

During the course of preparing acylated variants of oleandomycin, the novel crystalline, biologically-active^{500,544} triacetyloleandomycin was isolated by Celmer, Els and Murai,⁵⁰⁵ The triacetate is very insoluble in water, and therefore lacks the bitter taste characteristic of macrolide antibiotics. Triacetyloleandomycin is the result of acetylation of the hydroxyl group in the desosamine (LXXVI) R_1 , *L*-oleandrose (LXXIX) R_2 , and oleandolide R_3 (macrolide nucleus). By selective acetylation and deacetylation reactions, Celmer and Hochstein⁵⁴¹ prepared all the possible partially acetylated variants of oleandomycin. Tests for detecting the acetyl substituent on R_1 depended on low pK'_a values (6.6) and characteristic absorption at 9.5μ ; for acetylation of R_2 a positive violet Keller-Kiliani test was used; and for R_3 a positive Cotton effect rotary dispersion curve resulted when the hydroxyl in position *beta* to the ketone was acetylated. This result is in contrast to a negative-Cotton-effect rotary dispersion curve observed for oleandomycin and its esters not substituted in the nucleus.^{541,545}

The three diacetyl and three monoacetyl derivatives are all biologically active and, in addition, exhibit the characteristic ketonic ultraviolet absorption maxima at $290 m\mu$ of oleandomycin. Some of these variants can be recovered from a saturated solution of triacetyloleandomycin in water. The sequence involves first, hydrolysis of the acetyl on the desosamine (R_1) followed by a much slower deacetylation of the *L*-oleandrose (R_2) substituent. The resulting monacetyl derivative tenaciously retains the oleandolide (R_3) acetyl group; this product is more stable than

oleandomycin itself. Chemically, the acetyl substituents are introduced in the same order as they are removed $R_1 > R_2 > R_3$; however, the sequence can be altered by special catalytic deacetylations: $R_2 > R_1 > R_3$. A study of metabolic products in urine following oral ingestion of the $R_1R_2R_3$ triacetyl-, R_2R_3 diacetyl-, and the R_1R_3 diacetyloleandomycin derivatives revealed different orders of deacetylations from those obtained chemically, namely $R_3 > R_2 > R_1$ and $R_2 > R_3 > R_1$.⁵⁴⁶

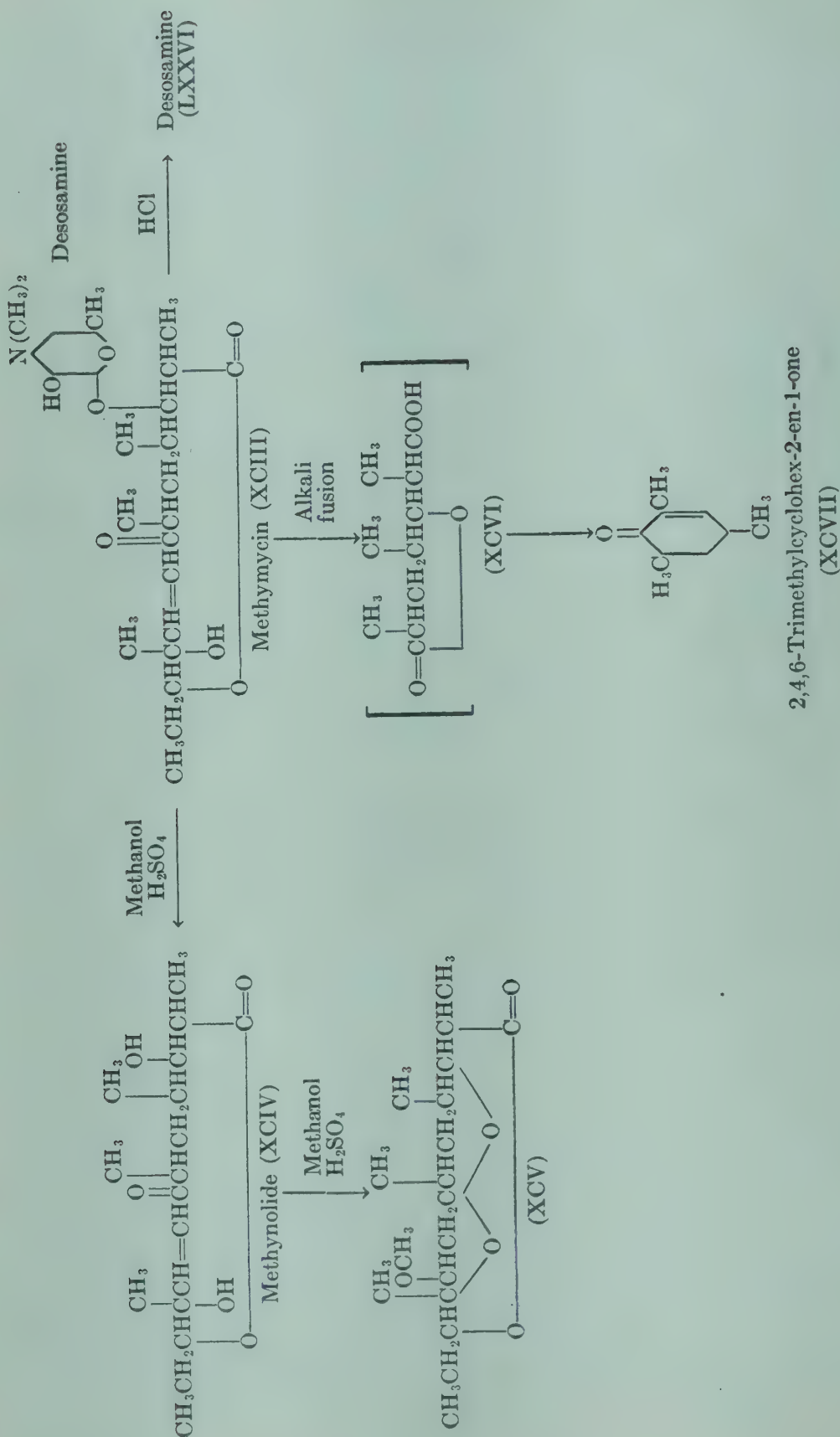
4. METHYMYCIN

Methymycin was extracted and crystallized by Donin, Pagano, Dutcher, and McKee from broths fermented with a *Streptomyces* found in a soil sample taken in Oswego, New York.²¹⁶ The basic antibiotic with the molecular formula $C_{25}H_{43}NO_7$, exists in at least two crystalline polymorphic forms: needles, m.p. 203–205°²⁵⁷ and prisms 195.5–197°.²¹⁶ They possess, however, the same ultraviolet and infrared spectrum and the same rotation: $[\alpha]_D + 61$ (in methanol).^{216,257} Methymycin forms crystalline salts e.g., the sulfate, m.p. 170–173°; and the hydrogen sulfate,²¹⁶ 2,4-dinitrophenylhydrazone, diacetyl, and N-oxide derivatives have helped to characterize the antibiotic.²⁵⁷ It can be hydrogenated in ethanol with palladized charcoal to dihydromethymycin: m.p. 193–195°.^{216,257} Further reduction with sodium borohydride yields tetrahydromethymycin: m.p. 169–174°.²⁵⁷ Hydrogenation of methymycin in glacial acetic acid in the presence of platinum oxide gives tetrahydrodeoxymethymycin: m.p. 173–175°.^{216,257}

Dihydromethymycin lacks the ultraviolet absorption band associated with the α,β -unsaturated carbonyl system present in methymycin itself,²¹⁶ but shows, rather, the presence of an ester or lactone carbonyl, and the saturated carbonyl group produced by reduction of the double bond.²⁵⁷ Formation of tetrahydromethymycin involves hydrogenation of the double bond and the carbonyl group, but the latter is not implicated in the removal of an oxygen atom during hydrogenolysis to form tetrahydrodeoxymethymycin.

Joint proposals by Djerassi, Zderac, Bowers, Khastgir, Hodges, and Riniker^{257,230,258,221} have led to structure XCIII as the complete expression for methymycin. While this antibiotic presents no exception to the complex structures associated with this class of substances, certain explicit experiments provided valuable clues to its unique structure.

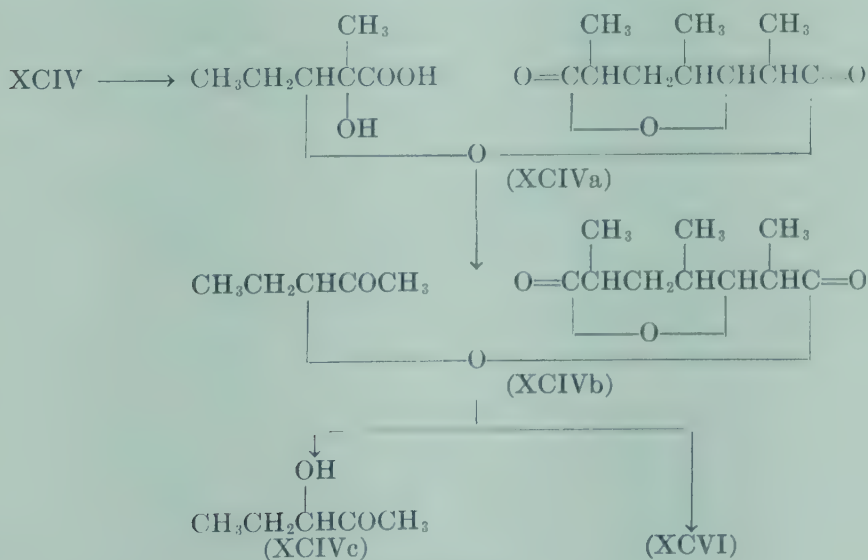
After several trials methymycin was made to liberate a high yield of desosamine hydrochloride (LXXVI) during brief heatings of the antibiotic in 5 N hydrochloric acid²³⁰ (Scheme 16). Mild hydrolysis in methanol-sulfuric acid yields the desosamine-free fragment methynolide (XCIV). When methymycin or the lactone (XCIV) is treated with methanolic



Scheme 16

sulfuric acid, the spiroketal (XCV) is formed, which upon lithium aluminum hydride reduction produces the corresponding diol.²³⁰ This substance gave further support for the structure of methymycin, insofar as the relation of the hydroxyl groups to the carbonyl function is concerned.²²¹ One of the two hydroxyl groups in methynolide (XCIV) was found to acetylate readily in pyridine-acetic anhydride. Although the hydroxyl group of methynolide in the intact methymycin molecule is resistant to oxidation with chromium oxide, reaction of the methynolide monacetate with this reagent converts an hydroxy to a keto group to give a diketolactone. This evidence suggested that the desosamine residue was linked through the reactive hydroxyl group. Further, this hydroxyl group was placed in β -position to the lactone carboxyl, since the diketolactone obtained by chromium oxide oxidation was readily decarboxylated.²³⁰

Alkali fusion of methymycin²⁵⁸ at 260° produced an oil which was collected by distillation into a solution of 2,4-dinitrophenylhydrazine. The product was shown to be the hydrazone of 2,4,6-trimethylcyclohex-2-en-1-one (XCVII) subsequently established by synthesis. Although this product is not present *per se* in methymycin, it became the most important substance in the degradation studies. In attempts to explain the existence of the fusion product, it was deduced by a series of resourceful inferences that XCVII had arisen by a cyclization and cleavage process from a fragment containing the carbon sequence of XCVI. The correct structural features of the lactonic acid (XCVI) were supplied by identification of a series of oxidation products derived from XCIV (Scheme 16a). Mild permanganate oxidation of XCIV gave XCIVa which was oxidized further with lead tetraacetate to a ketodilactone (XCIVb). On alkaline



Scheme 16a

hydrolysis XCIVb produced pentane-3-ol,2-one (XCIVc) and XCVI. Thus it became clear that XCVI obtained also by alkali fusion,²⁵⁸ was derived from the same carbon atoms 1 to 7 of the methynolide carbon chain. The structure of the oxidation product XCVI was further confirmed by comparing it with the same acid derived from narbomycin and pikromycin.²⁵⁹

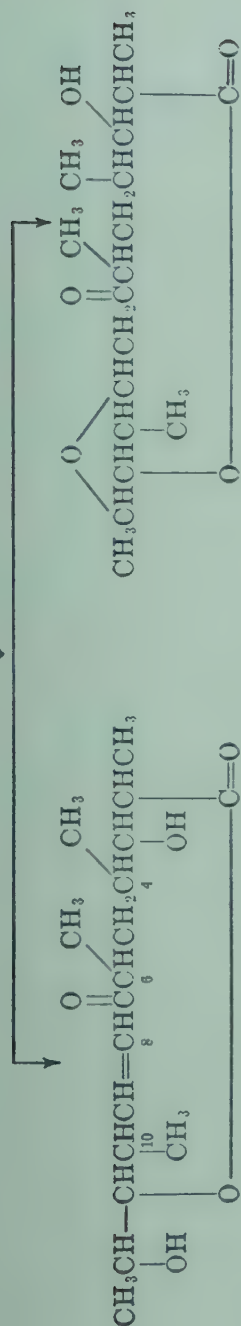
a. Neomethymycin. The correct structural alternative for methymycin (XCIII) itself has been substantiated by the work of Djerassi and Halpern on neomethymycin (XCVIIa),²²⁵ a second antibiotic isolated from fermentation mother liquors of methymycin. It was soon recognized that neomethymycin differs from methymycin only in the position of an oxygen atom in the aglycone portion of the molecule²²⁵; the position of the OH group at C-10 and an atom of H at C-12 are interchanged. Cleavage of neomethymycin with dilute sulfuric acid produces two products, neomethynolide (XCVIIb) and cycloneomethynolide (XCVIIc) (Scheme 16b). Ozonolysis of XCVIIb gives the lactonic acid XCVI, obtained previously by permanganate oxidation of methymycin,²³⁰ narbomycin,⁴⁷⁰ and pikromycin⁴⁷⁰; thus establishing the C-1 to C-7 carbon chain in neomethymycin present in methymycin.

Cycloneomethynolide (XCVIIc) lacks the unsaturated carbonyl chromophore of XCVIIb. A new ether linkage appears which is formed by addition of the C-12 hydroxyl group to the double bond of the α,β -unsaturated ketone. These and other fragments from the degradation of neomethymycin formed the basis for explaining the slight differences in the structure of these closely related antibiotics.²²⁵

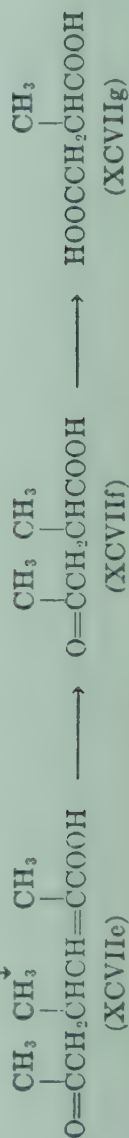
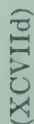
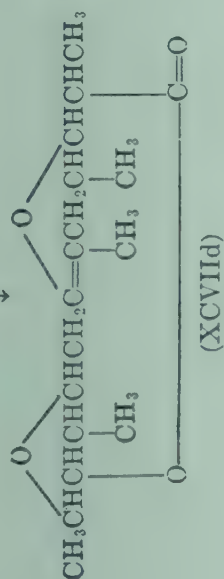
The absolute configuration of C-4 and C-6 of methymycin and neomethymycin has been related experimentally to *L*-glyceraldehyde by Djerassi and Halpern⁴⁶⁹ through an interesting series of reactions as shown in Scheme 16b, starting with the cyclic enol ether (XCVIIId) obtained from XCVIIb or XCVIIc when either was shaken with acidified ether and distilled at 40 mm and 200°. Purification of the products obtained by ozonolysis of XCVIIId yielded 6-keto-2,4-dimethyl-2-heptenoic acid (XCVIIe). Ozonolysis of XCVIIe followed by permanganate oxidation produced α -methyllevulinic acid (XCVIIIf). Hypoiodite oxidation of XCVIIIf furnished iodoform and (–)-methylsuccinic acid (XCVIIg). Since XCVIIg has been related to *L*-glyceraldehyde,⁴⁷¹ the absolute configuration of C-4 in neomethymycin is established.⁴⁶⁹ Further, neomethymycin and methymycin have been transformed to identical lactones (XCVI) which still retain the C-4 and C-6 substitutions and orientations of the parent antibiotics. Since XCVI has been converted into *meso*- α,α' -dimethylglutaric acid, the absolute configurations of C-4 and C-6 in methymycin and neomethymycin have been established as related to *L*-glyceraldehyde.⁴⁶⁹

Neomethymycin Degradation

Neomethymycin (XCVIIa)


$$\text{Neomethynolide (XCVIIb)} \xrightarrow{\text{O}_3} \text{XCVI}$$


Cycloneomethynolide (XCVIIc)



Scheme 16b

(XCVIIg)

5. SPIRAMYCIN

Another member of the macrolide group of antibiotics, spiramycin^{508,509,515,516} was extracted from culture filtrates of *Streptomyces ambofaciens* by Pinnert-Sindico, Ninet, Preud'homme, and Cosar.⁵⁰⁸ The sample of soil fungus was found in the Perrone region of northern France. This species of fungus produces a second antibiotic, congocidin.^{515,517} Spiramycin is easily separated from congocidin by methyl ethyl ketone extraction of culture filtrates adjusted to pH 9.⁵⁰⁸ Spiramycin base $[\alpha]_{20}^D - 80^\circ$ is slightly soluble in water and soluble in most organic solvents. The sulfate salt is soluble in water and methanol. An aqueous solution of the base containing disodium phosphate can be separated by countercurrent distribution with cyclohexane into three components, two of which were found to be almost identical.⁵⁰⁸

Spiramycin I ($C_{45}H_{78}N_2O_{15}$), spiramycin II ($C_{47}H_{80}N_2O_{16}$), and spiramycin III ($C_{48}H_{82}N_2O_{16}$)⁵¹⁸ were found to be identical with formacidins A, B, and C isolated from culture filtrates of a streptomycetes related to *Streptomyces ambofaciens*. Corbaz and co-workers⁵²¹ showed that mild hydrolysis of the formacidins A, B, and C gave mycrase (LXXVIII) a dimethylamino sugar, and forocidins A, B, and C.

On cleavage of spiramycins I, II, and III at pH 4 at 35° for ten days, Paul and Tchelitcheff^{518,519,520} obtained mycarose and neopsiramycins I, II, and III. At 80° the neopsiramycins are hydrolyzed into different non-antibiotic forocidins⁵²¹ and mycaminose (LXXV) which is also derived from carbomycin.²²⁶ The more difficult assignment of the ring system has not yet been accomplished.

G. NOVOBIOCIN

Novobiocin is still another example of a case of several independent announcements of the same antibiotic. It was isolated and named differently by three distinct groups of investigators: cathomycin (Merck)²⁶⁰; streptonivicin (Upjohn),²⁶¹ and cardelmycin (Pfizer).²⁶² Considerable confusion in terminology exists until an antibiotic is characterized, and investigators have an opportunity to compare their respective substances. In one case novobiocin was confused²⁶³ with albomycin, an antibiotic discovered by Soviet workers,²⁶⁴ because albomycin resembles an American trade name (abamycin) for novobiocin.

The Merck group called their novobiocin-producing actinomycete, *Streptomyces spheroides*²⁶⁰; and Upjohn, *Streptomyces niveus*,²⁶¹ while Pfizer used a non-specified *Streptomyces*.²⁶⁵ Various groups have demonstrated the *in vitro* activity of the antibiotic against certain gram-negative microorganisms^{266,267,268,269,270} e.g., the *Proteus* group.²⁶⁸ In addition,

several investigators have shown its effectiveness in experimentally induced infections,^{269,271,272,273} and in the treatment of humans^{266,272,274,275} particularly in infections caused by antibiotic-resistant *Micrococcus pyogenes*.^{266,267,276,277,278}

The pale yellow antibiotic was first isolated by Kaczka, Wolf, Rathe, and Folkers²⁷⁹ in two crystalline forms: melting points 152–154° and 170–174°; and subsequently by Hoeksema, Johnson, and Hinman, m.p. 152–156°, and 174–178°.^{280,281} These forms have the same optical activity: $[\alpha]_D^{25} - 27^\circ$ (in 1 N sodium hydroxide)²⁷⁹; $[\alpha]_D^{25} - 63^\circ$ (in ethanol)²⁸¹ and identical ultraviolet spectra.²⁸¹ Earlier approximate formulas for novobiocin^{279,280,281} were later revised to $C_{31}H_{36}N_{20}O_{11}$.^{282,283} Physical and chemical characteristics showed novobiocin to be entirely different from the known classes of useful antibiotics.

Novobiocin forms a white crystalline monosodium salt, $[\alpha]_D^{24} - 38^\circ$ (in ethanol); a disodium salt; and a mono- and dicalcium salt.²⁸⁰ The antibiotic combines also with many common basic antibiotics to form water insoluble salts.²⁸⁴

The Merck^{279,283,285,286} and Upjohn groups^{281,282,287} worked independently to establish the unique structure of novobiocin (XCVIII) which has been named 7-[4-(carbamoyloxy)-tetrahydro-3-hydroxy-5-methoxy-6,6-dimethylpyran-2-yloxy]-4-hydroxy-3-[4-hydroxy-3-(3-methyl-2-butenyl)-benzamido]-8-methylcoumarin.²⁸⁷ Substantially complete structures for novobiocin were given first by Shunk, Stammer, Kaczka, Walton, Spencer, Wilson, Richter, Holly, and Folkers^{285,497} and subsequently by Hoeksema, Caron, and Hinman.^{287,496}

The glycoside bond of novobiocin is cleaved with methanolic hydrogen chloride to give the crystalline methyl glycoside of 3-carbamyl-4-methyl-novobiose (XCIX) and cyclonovobiocic acid (C) (Scheme 17). The sum of the formulas of the methylglycoside and the cyclonovobiocic acid, less one molecule of methanol, add up to the formula $C_{31}H_{36}N_{20}O_{11}$ for novobiocin.²⁸³ Further degradation of C in aqueous sodium hydroxide yields 2,2-dimethyl-6-carboxychroman (CI)^{281,282,283} confirmed by comparison with a known sample.²⁸⁸ The precursor of CI, 5-hydroxy-3-(3-methyl-2-butenyl) benzoic acid (CII), also established by synthesis, can be obtained directly from novobiocin by hydrolysis in sodium hydroxide.²⁸³ It was shown that the 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic acid (CII) is attached to the coumarin moiety²⁸¹ through an amide linkage.²⁸⁵ The structure of cyclonovobiocic acid as given by C has been confirmed through synthesis by Spencer, Stammer, Rodin, Walton, Holly, and Folkers.²⁸⁶

Hydrogenation of novobiocin with platinum oxide or Raney nickel catalysts yields dihydronovobiocin (CIII),^{281,283} thus converting the

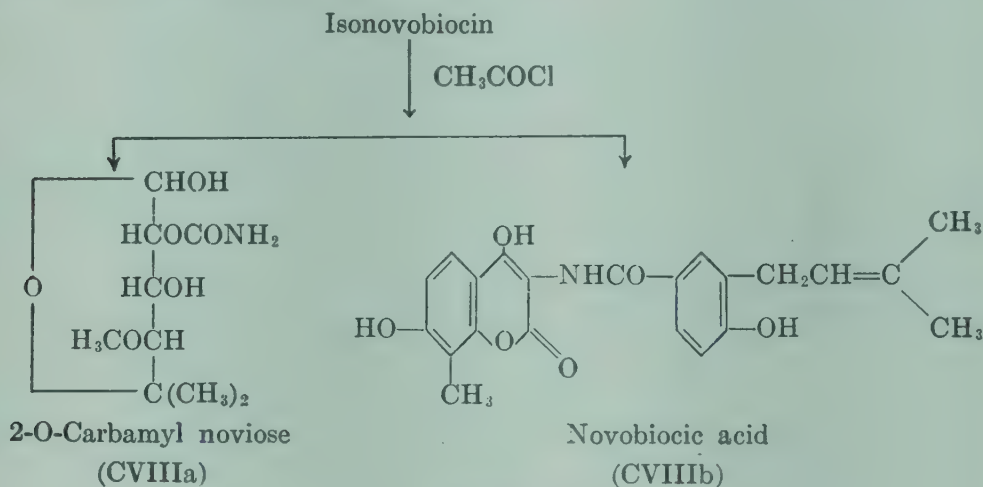
3-methyl-2-butenyl group into an isopentyl chain. The dihydro derivative has a biologic activity comparable to that of novobiocin itself.²⁸³ With aqueous alkali, dihydronovobiocin can be cleaved into 4-hydroxy-3-isopentyl-benzoic acid; however, hydrolysis with hydrochloric acid in methanol yields dihydronovobiocic acid (CVI). Treatment of CVI with hydrogen bromide-acetic anhydride mixture gives the diacetylated derivative (CVII). Successive deacetylation by dilute alkali and dilute hydrochloric acid-dioxane yields the aromatic amine hydrochloride (CVIII).^{285,497} The structure of CVIII was confirmed by comparing it with the demethyl analog of CVIII, synthesized through 2,4-dihydroxy-3-methylbenzoic acid. The infrared absorption spectrum of the demethyl analog was essentially identical with CVIII. Location of the 7-hydroxyl and 8-methyl groups was established by degradation of CVIII to known compounds²⁸² and thus the structure of the coumarin moiety was complete.^{282,285,496,497} Verification of the complete structure assigned to dihydronovobiocic acid (CVI) was provided by synthesis of CVII.²⁸⁶ Spencer, Rodin, Walton, Holly, and Folkers⁴⁹⁸ synthesized novobiocic acid (CVIIIb), dihydronovobiocic acid (CVI), and cyclonovobiocic acid (C) by acylation of 3-amino-2,7-dihydroxy-8-methylchromone with the appropriate benzoyl chlorides.

At this point, only the structure of the sugar moiety and its linkage to the aglycon moiety had to be assigned.^{285,287,289} The presence of the urethane group in XCIX was inferred from its infrared spectrum. Moreover, alkaline hydrolysis of XCIX splits out carbon dioxide and ammonia and yields methyl-4-methylnovobioside.²⁸⁵

The methyl glycoside of XCIX does not react with sodium periodate. Hydrolysis with dilute hydrochloric acid, however, produces 3-carbamyl-4-methylnovobiose (XCIX) which reacts with one mole of periodate.⁴⁹⁶ The reaction reveals the presence of an hydroxyl group α to the glycosidic carbon of XCIX. Reaction of XCIX with ethyl mercaptan and hydrogen chloride produced the mercaptal derivative, which was reduced with Raney nickel and hydrolyzed to yield CIV. Periodate oxidation of CIV, followed by bromine oxidation, gave acetaldehyde and $(-)\alpha$ -methoxy- β -hydroxyisovaleric acid (CV). These products furnished cogent evidence for the structure of the sugar.^{285,287,497} Based on these data and on the rules of optical activity Walton, Rodin, Stammer, Holly, and Folkers²⁸⁹ assigned the configuration of *L*-lyxose to XCIX. Evidence was provided by both groups of investigators who showed that the sugar moiety, noviose (XCIX) is attached glycosidically to the 7 position of 3-[4-hydroxy-3-(3-methyl-2-butenyl)-benzamido]-4,7-dihydroxy-8-methylcoumarin (C).^{282,287} The sugar moiety is the only fragment of the novobiocin molecule (XCVIII) which has not yet been synthesized; otherwise the

structure of the antibiotic elucidated by degradative studies has been amply confirmed by synthesis.^{286,498}

When a solution of novobiocin was held at pH 10 for two hours at room temperature, bioassays of the solution indicated 30–35% loss of antibiotic activity. The apparently homogeneous material was resolved by counter-current distribution into two main components after 2000 transfers. Analysis of the distribution data showed the mixture to contain about 67% novobiocin (XCVIII) and 33% of a second but inactive component. Hinman, Caron, and Hoeksema⁴⁹⁵ showed that under these conditions, novobiocin undergoes an interesting isomerization into the biologically inactive isonovobiocin. This isomer was cleaved in methanol-acetyl chloride (Scheme 17a) to crystalline novobiocic acid (CVIIIb)



Scheme 17a

identical with that obtained from novobiocin⁴⁹⁷ under these conditions, and the methyl glycoside of 2-0-carbamyl-4-0-methyl-5,5-dimethyl-*L*-lyxoside (CVIIIa)⁴⁹⁷ isomeric with the sugar (XCIX) isolated from novobiocin. It is interesting that Spencer and co-workers⁴⁹⁸ found that novobiocic acid was also obtained directly from novobiocin on treatment with sulfuric acid in dioxane.

H. POLYENE ANTIBIOTICS

Since 1950, an interesting class of antifungal antibiotics has been discovered among the products of *Streptomyces*. They bear closely similar chemical properties, and contain a common conjugated polyene chromophore, as evidenced by characteristic ultraviolet absorption spectra.^{290,291,500} Attempts have been made to classify these antibiotics into four subgroups depending on the presence of a polyene moiety containing four, five, six or seven conjugated double bonds.^{290,291,292} Representative of this fairly

large group are nystatin²⁹³ and rimocidin,²⁹⁴ two antibiotics with particularly useful properties. They contain a tetraene system and also have identical absorption maxima as shown in Table 2-3.

TABLE 2-3
REPRESENTATIVE POLYENE ANTIBIOTICS

Antibiotic	Absorption Maxima (m μ)		
Nystatin	290	304	319
Rimocidin	291	304	318
Fumagillin	322	336	351

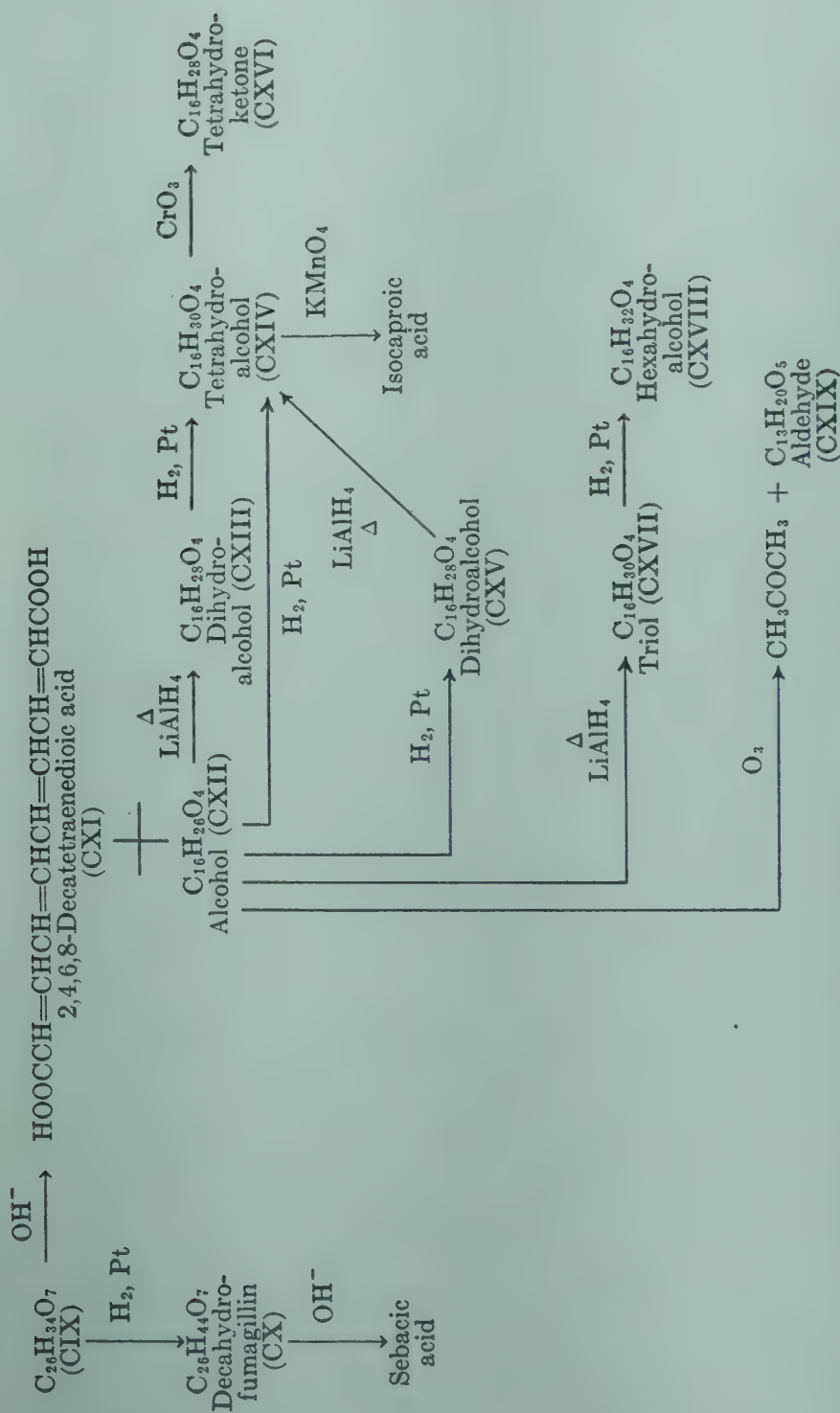
While fumagillin²⁹⁵ is classified as a polyene antibiotic, it differs conspicuously from other substances of this group in exhibiting the effect of a conjugated carbonyl system in its absorption spectrum (Table 2-3), and in its antibiotic spectrum.

1. FUMAGILLIN

Fumagillin (H-3) is a potent amebicide,²⁹⁶ but not much importance was attached to its earlier discoveries by Asheshov, Strelitz, and Hall,²⁹⁷ and by Hanson and Eble,²⁹⁸ two independent groups who found it capable of inhibiting bacterial viruses. The antibiotic was isolated from cultures similar to *Aspergillus fumigatus*.^{296,299} Fumagillin has little antibacterial and antifungal activity, and no antiviral activity when tested in mice with M M virus and influenza PR8A infections.²⁹⁸ In contrast to other amebicides, it would appear that fumagillin exerts its action directly on *Endamoeba histolytica*.^{295,297,300,301}

The crystalline form of the antibiotic was isolated and characterized by Eble and Hanson²⁹⁵; however, some time later, white crystalline needles of higher purity were obtained³⁰²; m.p. 194–195°, $[\alpha]_D^{25} - 26.2^\circ$ (in ethanol). The antibiotic forms a methyl ester^{295,302} and a monopotassium salt.²⁹⁵ Fumagillin is soluble in most organic solvents and in dilute alkaline solutions, but is insoluble in saturated hydrocarbons, water, and dilute acids. Earlier approximate empirical formulas for fumagillin^{295,303} have been more exactly stated as C₂₆H₃₄O₇ (CIX).³⁰⁴ In the presence of a platinum catalyst, fumagillin absorbs about five moles of hydrogen per mole to form decahydrofumagillin (CX) (Scheme 18), which on hydrolysis with two equivalents of base produces sebacic acid and other products.³⁰³

Under mild alkaline conditions the intact antibiotic is cleaved smoothly to yield an unsaturated acid, 2,4,6,8,-decatetraenedioic acid (CXI), probably the first time this acid has been isolated from a natural source. It has been shown by Brown and Landquist³⁰⁵ and by Tarbell,



Scheme 18

Al-Kazimi, Hoffman, Page, Vogt, Hargie, Isarasena, Ross, Wargotz and Schenck^{302,303,304} that fumagillin is the mono-ester of the acid CXI and an alcohol, $C_{16}H_{26}O_4$ (CXII) of a complex structure^{295,301,303,559} (Scheme 18). The alcohol (CXII) resisted crystallization for several years, but crystals were finally obtained by careful development of alumina chromatograms, m.p. $55.5\text{--}56^\circ$; $[\alpha]_D^{23} - 68.0^\circ$ (in ethanol).³⁰⁶ Analyses of the alcohol (CXII) indicate that it contains one methoxyl, a secondary hydroxyl, two C-methyl groups, and two non-carbonyl oxygen systems.³⁰⁴ In addition, chemical evidence has been obtained for the presence in CXII of an epoxide ring and a carbon-carbon double bond. The epoxide ring can be opened on mild reduction with lithium aluminum hydride to give the crystalline dihydroalcohol $C_{16}H_{28}O_4$ (CXIII), m.p. 53° , containing a new hydroxyl group. The double bond in CXIII can be reduced catalytically to a crystalline tetrahydroalcohol derivative, $C_{16}H_{30}O_4$ (CXIV), m.p. $89\text{--}90^\circ$,³⁰² which contains two hydroxyl groups, one secondary and one tertiary. Alternatively, the double bond in CXII can be reduced first to give the dihydroalcohol (CXV), $[\alpha]_D - 50^\circ$, which is different from the dihydroalcohol (CXIII). Subsequent reduction of CXV with lithium aluminum hydride yields a tetrahydroalcohol identical with CXIV. Catalytic reduction of the alcohol (CXII) with platinum yields directly, in one step, tetrahydroalcohol (CXIV) in which the double bond is reduced and the epoxide group is hydrogenolyzed.³⁰⁶ Oxidation of the tetrahydroalcohol (CXIV) with chromium trioxide in pyridine produces tetrahydroketone $C_{16}H_{28}O_4$ (CXVI).³⁰² It forms, on treatment with furfural and aqueous alkali, a crystalline furfurylidene derivative, m.p. $96\text{--}96.7^\circ$. It appears that the carbonyl oxygen in CXVI corresponds to the hydroxyl of the alcohol (CXII) which is esterified in fumagillin.³⁰⁶

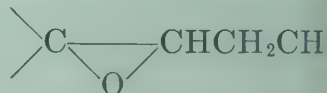
There appears to be no rearrangement involved in the transformation of CXII to the ketone (CXVI).⁵⁵⁰ Ozonizations of the furfurylidene derivative of CXVI and treatment of the ozonide with hydrogen peroxide yields a carboxylic acid containing a γ -lactone group. Since the product analyzed for a $C_{16}H_{26}O_6$ molecular formula, demonstrating that it contained all the carbon atoms originally present in the tetrahydroketone (CXVI), it was deduced that the hydroxyl group in the alcohol (CXII) itself is on a carboxyclic ring.⁵⁵⁰

While the mild treatment of lithium aluminum hydride on the alcohol (CXII) forms dihydroalcohol (CXIII), more vigorous action of the reagent on CXII in boiling tetrahydrofuran opens both the epoxide ring and a second cyclic ether to yield a triol, $C_{16}H_{30}O_4$ (CXVII). The side chain double bond in the triol (CXVII) can be reduced readily by platinum and hydrogen to give a hexahydroalcohol, $C_{16}H_{32}O_4$ (CXVIII)^{304,306}. The hexahydroalcohol (CXVIII) can be dehydrogenated with selenium and

with palladium-on-charcoal. The volatile product of this reaction forms the dinitrophenylhydrazone of ethyl isoamyl ketone.³⁰⁴

The alcohol (CXII) is highly reactive to acid and base. On heating with sodium hydroxide in aqueous dioxane, it yields three products: two monoöls, $C_{16}H_{26}O_4$, isomers of CXII, and a triol $C_{16}H_{28}O_5$ in a total yield of about 60%. The monoöls no longer contain the exopide ring present in their precursor alcohol. They are regarded as being formed by attack of the hydroxyl group on the epoxide linkage of CXII, with formation of a new oxygen-containing ring and generation of a new hydroxyl group. The triol contains one ether ring which may be the original one present in CXII and a secondary hydroxyl group. Each of the three products is reduced catalytically to form a crystalline dihydro derivative.⁵⁵¹

Aqueous acids, particularly oxalic and sulfuric, convert the alcohol (CXII) into a complicated mixture of isomerization and hydration products. All the products are diols or triols with no carbonyl groups.⁵⁵² These experiments on acid treatment of CXII did not provide clear-cut support for the presence of the suspected side chain



$=\text{C}(\text{CH}_3)_2$,^{306, 552, 554} because of the great tendency of CXII to undergo rearrangement under hydrating conditions.⁵⁵²

A vast amount of degradative work has been done on the complex alcohol (CXII) hydrolyzed from fumagillin; however, only limited conclusions on the nature of its complex structure have been drawn from these experiments. For example, oxidation of the tetrahydroalcohol (CXIV) with permanganate in dilute sulfuric acid yields insocaproic acid which

gives evidence for the presence of $-\text{CCH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$ in the mole-

cule.³⁰² The presence of the group $-\text{CCH}_2\text{CH}=\text{CH}(\text{CH}_3)_2$ in the alco-

hol (CXII) has been deduced from ozonization of CXII which cleaved the molecule into acetone and an α,β -unsaturated aldehyde (CXIX).

The experiments described do not permit of even a tentative formula for the alcohol moiety (CXII) of the fumagillin molecule. The alcohol appears to contain an unsaturated side chain, a six-membered carbocyclic ring and perhaps two oxygen-containing rings.³⁰⁴

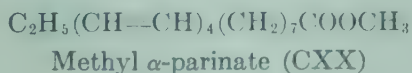
2. NYSTATIN

During the course of a search for actinomycetes as antagonists to pathogenic fungi, an antibiotic substance called fungicidin (nystatin) was

isolated by Hazen and Brown³⁰⁷ from fermentation of a strain of *Streptomyces noursei*^{307,308} found in a farm soil in Virginia. The microorganism produced two antibiotics in broth culture. The readily soluble one was identified as cycloheximide (XXIX).^{113,114,116,307} The second antibiotic, which appeared in good yield in the mycelium, was found to have both fungistatic and fungicidal activity.³⁰⁷ The crude concentrate was used to determine the antifungal spectrum *in vitro*, and its efficacy in treating mice infected with histoplasmosis.^{307,309} cryptococcosis,³⁰⁷ coccidioidomycosis,³¹⁰ and moniliasis.^{311,312} In addition, the antibiotic was found to protect mice from an otherwise lethal mixture of *Candida albicans* and chlortetracycline.³¹¹ In humans, it shows very low toxicity, and is effective in monilial infections.^{312,313}

Crude preparations were found to be sparingly soluble in methanol and ethanol and relatively insoluble in water. Because the antibiotic could be ultrafiltered through fine gradocol membranes, it was thought to have a relatively small molecular weight.³⁰⁷ The material was crystallized first by Brown, but a highly purified nystatin obtained by the slow evaporation of a biphasic system made up of n-butanol, methanol, water, and hexane gave an analysis for the molecular formula $C_{46}H_{83}NO_{18}$.²⁹³ Extensive analyses of crystalline fine needles were found in good agreement by Dutcher, Walters and Wintersteiner for the molecular formula of $C_{46}H_{77}NO_{19}$.³¹⁴ The antibiotic decomposes above 165° without melting even at 250°, and shows $[\alpha]_D^{25} + 21^\circ$ (pyridine).^{293,314} On addition of exactly one equivalent of sodium hydroxide, the biologically active sodium salt is formed.³¹⁴

The ultraviolet absorption maxima of nystatin are given in Table 2-3.³¹⁵ In ethanol part of its spectrum is almost identical with methyl α -parinate, (CXX) which contains four conjugated double bonds.^{291,314,316}



Comparison of both spectra suggests that the antibiotic contains the same chromophore.

Apart from the evidence for the conjugated tetraene system, the ultraviolet absorption confirms the presence of a conjugated diene system as well. In the presence of either platinum or palladium catalysts, nystatin absorbs six moles of hydrogen per mole, thus saturating six carbon-carbon double bonds. The resulting dodecahydronystatin exhibits only end absorption in the ultraviolet and no antifungal activity.

Infrared absorption of nystatin reveals a broad intense hydroxyl band, a carbonyl band, which is probably due to a lactone group, and a carboxylate band. The carboxylate group is probably involved in the

formation of a monomethyl ester when the antibiotic is treated with diazomethane. Nystatin shows the presence of four C-methyl groups and nitrogen in the form of a primary amino group.³¹⁴

Since nystatin is heavily hydroxylated, the presence of a carbohydrate moiety was inferred. Earlier unsuccessful attempts³¹⁴ led eventually to the isolation of mycosamine, an aminodeoxyhexose.³¹⁷ It is of interest that the identical amino sugar was isolated from amphotericin B,^{318,319} a polyene antifungal antibiotic,³²⁰ during structure studies by Dutcher, Young, Sherman, Hibbits, and Walters.³¹⁷

3. RIMOCIDIN

Along with oxytetracycline¹⁵⁶ culture broths of *Streptomyces rimosus* were found to contain an antifungal antibiotic, designated rimocidin.²⁹⁴ It was recovered from mycelium by extraction with n-butanol.

The ultraviolet spectrum of rimocidin is virtually identical with that of nystatin (Table 2-3); moreover, its chemical composition is very similar. However, these two antibiotics differ somewhat in their general biologic and chemical properties, but limited information is available. Rimocidin contains both basic and acidic groups. It forms a crystalline hydrated sulfate salt: $[\alpha]_D^{25} + 75.2^\circ$ (methanol); m.p. 151° (dec.), and a crystalline sodium salt.

I. THE POLYPEPTIDE ANTIBIOTICS

Because of its historical importance, penicillin leads this antibiotic review. Logically, however, the penicillins (I) should be described in this section, since they may be considered as bicyclic dipeptides, made up of the amino acids serine and *D*- β - β' -dimethyleysteine, with various acyl radicals attached to the serine moiety. In contrast to penicillins, the polypeptide antibiotics manifest higher toxicities; nonetheless more than a few have become significantly important. Naturally-occurring peptide antibiotics frequently contain uncommon amino acids which belong either to the *D*-series, or have not hitherto been found as constituents of ordinary proteins.³³⁰ In addition, they frequently contain unusual peptide linkages which hitherto have not been detected in proteins.

The new techniques for purifying polypeptides have contributed greatly to progress in this field. These advances have been achieved particularly by partition between water and organic solvents, notably by Craig countercurrent distribution.²¹ Nonetheless, these more refined fractionation methods have lightened only slightly the arduous tasks of separating and ultimately purifying antibiotic components.

Despite the complexity of these polypeptides, the natural course of

investigation is to identify the amino acid composition of these antibiotics, and if possible, determine their arrangement and sequence in the polypeptide. An important advance in determining the succession of amino acids in polypeptide chains was made by Sanger³³¹ in 1945. The procedure involves partial hydrolysis, subsequent separation of smaller peptides, and a determination of sufficient structures until an over-all sequence is established. The "end group analysis" method consists in treating peptides with 2,4-dinitrofluorobenzene, which labels the terminal amino group. The newly-formed bond is stable to acid, thus the peptide can be subjected to partial hydrolysis to give a mixture of smaller residues, which can be identified as their yellow dinitrophenyl derivatives. The so-labeled terminal peptide fragment can be degraded further to its component amino acids.

Fractionation of amino acids obtained from complete hydrolysis or from partial hydrolysis techniques is extremely laborious. Consequently, many ingenious innovations have been devised to overcome technical difficulties in the separation, purification, and identification of the constituent amino acids. A great impetus was provided to amino acid research with the development of chromatographic methods. Following the work of Neuberger,³³² in which the hydrolysis products of egg albumin protein were reacted with ketene, Synge³³³ separated acetylated amino acids in packed columns of silica gel which provided the inert support for the aqueous phase, while the organic phase was allowed to flow through the column. Partition chromatography was greatly improved by Martin and Synge⁵⁶⁰ and by Consden, Gordon, and Martin in 1944³³⁴ who developed a variety of paper-strip chromatographic techniques. The amino acid hydrolyzates are partitioned between water, absorbed on paper and thus retained in a stationary phase, and a partly immiscible organic solvent which flows along the length of the strip and so sets up a moving phase. Boundary anomalies interfere less in zone electrophoresis, more commonly employed using filter paper strips.³³⁵ By this technique, which depends essentially on the differences in charge, it is possible to obtain separation of components into zones of migration. Chromatographic fractionation on starch columns, as devised by Moore and Stein,³³⁶ has been improved greatly by their ion-exchange chromatography, which can be made to separate hydrolyzates from small quantities of amino acids with practically quantitative recoveries. The fractionation of these complex mixtures has been aided still further by an excellent supplementary method devised by Craig and King³³⁷ which makes use of multiple dialysis.

While only limited attempts have been made to synthesize polypeptide antibiotics, the outlook for such successful achievements is indeed

promising. The recent synthetic preparative methods³³⁸ devised for polypeptides are no less distinguished than the brilliant synthesis of glycylglycine by Fisher and Fournau³³⁹ in 1901, and the great contribution of Bergmann's³⁴⁰ carbobenzoxy method to peptide synthesis. In recent years, a number of other blocking groups has been proposed, of which the phthalylated derivatives developed by Sheehan,³⁴¹ and by King and Kidd³⁴² have been very useful. The extension of peptide chains has been greatly facilitated also by the use of the mixed anhydride method^{343,344,345} and by the direct carboxylic acid condensation method using tetraethylpyrophosphite, as proposed by Anderson.^{346,247,348}

1. TYROTHRIN: TYROCIDINES AND GRAMICIDINS

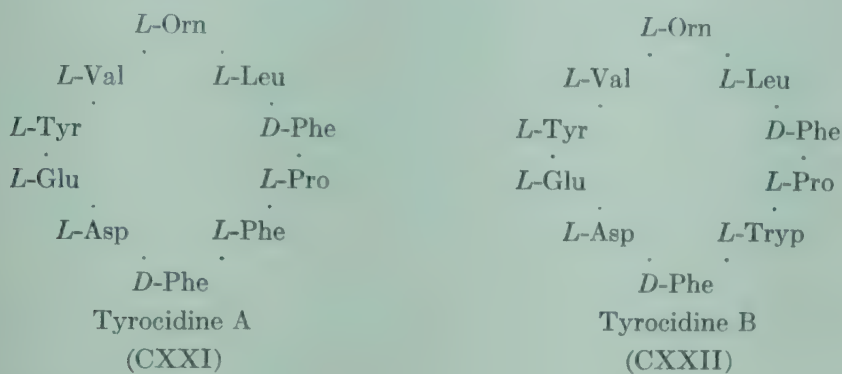
a. Tyrothricin

Tyrothricin, a mixture of polypeptide-like substances possessing antibacterial activity, was isolated from culture filtrates of strains of *Bacillus brevis* by Dubos^{349,350,351} during one of the earliest planned investigations to find a soil organism which would destroy pathogenic bacteria. Subsequent fractionation studies on autolyzed cultures by Hotchkiss and Dubos^{352,353,354} showed that tyrothricin could be separated into the neutral gramicidins and basic tyrocidines.³⁵⁴ Tyrocidine was recrystallized until its properties remained constant, thus satisfying the criteria of purity in use in 1940. However, these products were shown subsequently to be mixtures. Syngé and Tiselius^{355,356} attempted to fractionate the tyrocidines by carbon adsorption techniques, but later studies by Battersby and Craig³⁵⁷ using countercurrent distribution, showed that crystalline tyrocidine hydrochloride is composed of a family of polypeptides with three major components designated tyrocidines A, B, and C. The three peptides differ with respect to their tryptophan or tyrosine content. Tyrocidines B and C contain a large amount of tryptophan, whereas tyrocidine A contains none.^{357,358}

b. Tyrocidine A.

Tyrocidine A was isolated from the family of closely-related substances present in tyrocidine by multiple countercurrent distributions and crystallization from methanol-ether: m.p. 240–242°; $[\alpha]_D^{25} - 111^\circ$ (1:1 water-ethanol); $C_{66}H_{87}N_{13}O_{13} \cdot HCl$.³⁵⁷ The A-component has a molecular weight of about 1270, as obtained by Battersby and Craig³⁵⁹ from partial substitution studies. But two functional groups are present in tyrocidine A, namely, the hydroxyl group of a single tyrosine residue, and the δ -amino group of the ornithine moiety which accounts for the basicity of the molecule. It is a cyclic peptide³⁵⁸ with no free carboxyl group or

α -amino groups. Hydrolyzates of the antibiotic show the presence of valine, tyrosine, leucine, proline, ornithine, glutamine, asparagine and three phenylalanine molecules. Tyrocidine A was subjected to a series of partial hydrolyses, and a sufficient quantity of polypeptides was isolated, by countercurrent distribution and ion-exchange chromatography, to permit formulation of an unambiguous sequence for the amino acids in the molecule (CXXI).^{*360,362}



c. Tyrocidine B.

Tyrocidine B was recovered from the countercurrent distribution cuts obtained during the separation of the components. Tyrocidine B was purified by Craig and King^{358,362} by recrystallization from methanol-isopropyl ether mixtures and re-distributed countercurrently. This product gave an analysis corresponding to the molecular formula $C_{68}H_{88}N_{14}O_{13} \cdot HCl$.³⁵⁸ Only two functional groups were found in tyrocidine B, the δ -amino group of the single ornithine and the hydroxyl group of the tyrosine.³⁵⁸

The partial hydrolysis method which had worked so successfully in establishing the complete amino acid sequence for tyrocidine A, was found to be most unsatisfactory when applied to tyrocidine B.³³⁷ It was particularly difficult to isolate, for example, much, if any, peptide-containing tryptophan.³⁶² The separation of the peptides from partial hydrolysis of tyrocidine B in hydrochloric acid, was carried out by a series of fractionation procedures and aided by fractional dialysis with cellophane, which permits multiple fractional separations of mixtures of dialyzable solutes.^{337,362,363,364} King and Craig^{362,363} showed finally that tyrocidine B contains *L*-aspartic acid, *L*-glutamic acid, *L*-tyrosine, *L*-valine, *L*-ornithine, *L*-leucine, *L*-proline, *L*-tryptophan, and two moles of *D*-phenylalanine. It is likely that tyrocidine B, like tyrocidine A, is a cyclic ten-amino acid residue polypeptide (CXXII), a conclusion supported further

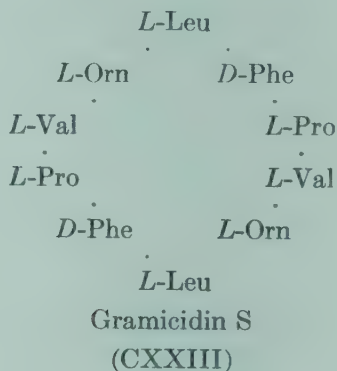
* In general the first three letters are used to designate the amino-acid residue.

by the absence of a free carboxyl group or α -amino group in the intact molecule. The optical configuration of the amino acid residues is the same in tyrocidine A and B.³⁶²

d. Gramicidins.

Along with the tyrocidines, the tyrothricin mixture contains at least four other closely-related substances, three of which have been crystallized and designated gramicidins A, B, and C. The gramicidins are neutral substances possessing neither free amino nor carboxyl groups³⁶⁵ and are characterized by the presence of 2-aminoethanol-1.^{366,367} Besides ethanolamine, gramicidin A shows the presence of *D*-leucine, *L*-tryptophan, *DL*-valine, *L*-alanine and glycine.^{365,368,369} Gramicidin B contains these amino acids and phenylalanine in addition, while gramicidin C contains tyrosine instead of phenylalanine.³⁷⁰

Gramicidin S ("Soviet gramicidin") is a polypeptide antibiotic elaborated by a different strain of *B. brevis*.^{371,372} However, the antibiotic closely resembles tyrocidine rather than gramicidin in its chemical and antibacterial properties. The molecule contains the non-natural amino acid *D*-phenylalanine, *L*-ornithine (which is seldom encountered in a naturally occurring peptide structure), *L*-valine, *L*-leucine and *L*-proline.³⁷³ The unit possesses two free amino groups contributed by the δ -amino group of the ornithine residue^{373,375,376} and no free carboxyl groups.³⁷⁶ The antibacterial substance is a cyclic decapeptide, or a twice repeated five amino acid sequence, in the form of two pentapeptides joined in a ring (CXXIII).³⁷³ This decapeptide structure (CXXIII)



has been established by the notable synthesis of gramicidin S by Schwyzer and Sieber in 1956^{377,378} and further supported by diffusion and cryoscopic measurements,³⁷⁹ by the countercurrent distribution of its 2,4-dinitrophenyl derivatives,³⁷⁴ and by X-ray examination of single crystals of a series of derivatives of gramicidin S.^{380,381,382} In addition, Erlanger, Curran, and Kokowsky⁵¹⁰ synthesized a decapeptide analog which differs

from gramicidin S in containing two *D*-tyrosine residues instead of two *D*-phenylalanines. Ring closure, by joining the proline and valine residues, and the antibacterial activity of the decapeptide has yet to be accomplished.

It has been suggested that the biologic activity of Gramicidin S depends upon its cyclic structure,³⁸³ but this theory has been challenged.³⁸⁴

2. THE BACITRACINS

Bacitracin, a mixture of polypeptide antibiotics, was isolated by Johnson, Anker, and Meleney³⁸⁵ in 1945 from cultures of *Bacillus licheniformis*, an organism of the *Bacillus subtilis* group. The name bacitracin was proposed in deference to a patient named Tracy, from whom a potent strain of the microorganism was isolated. Surface culture preparations of the antibiotic^{385,386} soon gave way to production from submerged cultures of *B. subtilis*.³⁸⁷ Independently, Newton, Abraham, and associates^{388,389} isolated the antibiotic mixture from a strain of *B. subtilis*, but designated the antibiotic "ayfivin".³⁹⁰ This name was withdrawn when it was shown that several components of ayfivin were similar to those in bacitracin,^{389,391,392} and further, that the amino acid composition of both antibiotics resembled each other.^{392,393,394}

Bacitracin has marked activity against gram-positive bacteria and little or none against gram-negative organisms.³⁸⁵ It shows some synergistic action with other antibiotics, particularly penicillin.^{397,398} Intensive purification of bacitracin yields preparations with a potency of about 60 units per mg.,³⁹⁴ but on storage, material of this purity declines in potency to a level of approximately 45 units per mg. Several attempts have been made to prepare more stable forms, such as zinc bacitracin³⁹⁵ and bacitracin methylene disalicylate.³⁹⁶

The antibiotic is labile and readily affected by alkali, strong acid, and formaldehyde.^{386,399,400} Even the mild methods used to separate the polypeptide mixture of antibiotics presented many difficulties and revealed their complex composition and instability. By the use of countercurrent distribution techniques,⁴⁰¹ Craig, Weisiger, Hausmann, and Harfenist⁴⁰⁰ demonstrated commercial bacitracin to be a mixture containing a main component, bacitracin A, and lesser amounts of bacitracin B, D, E, and F. In addition to these components, Newton and Abraham resolved crude bacitracin into at least ten polypeptides: E, D, B, A', A, C, G, F₁, F₂, and F₃.^{402,403} The A, B, D, and E components have ultraviolet absorption maxima at $\lambda = 253 \text{ m}\mu$; and F₁, F₂, and F₃ at 253 and 288 $\text{m}\mu$.⁴⁰³

It is reasonable to assume that bacitracin A, the main peak exhibited in countercurrent distribution studies, represents a single entity, but the

homogeneity of the other peaks is perhaps less certain, although it has been reported that bacitracin E has been obtained in a crystalline form.⁴⁰⁶ In its purified form, bacitracin A has been found to have a molecular weight of about 1,470, by the method of partial substitution which involves the reaction of bacitracin A with 1-fluoro-2,4-dinitrobenzene⁴⁰⁴, a molecular weight of about 1,460 by an ultracentrifugal method,⁴⁰⁵ and one of about 1500 by electrometric titration.⁴⁰⁶ These values suggest an molecular formula best represented^{404,407} by $C_{65}H_{103}H_{16}O_{17}S$ ($C_{65}H_{102-104}N_{16}O_{17}S$).

In neutral or slightly alkaline solution, bacitracin A is slowly transformed into bacitracin F.^{404,407} It is likely that other modifications, e.g. bacitracin E and bacitracin D, in this family of antibiotics are produced during recovery and separation of the antibiotic peptides. However, it appears that bacitracin C is biologically synthesized by certain strains of *B. licheniformis*. Its amino acid composition is similar to that of bacitracin A, but it contains a substance similar to a valine residue.^{403,411} It is probable that bacitracin C also contains a different amino acid residue which, it has been suggested, may be glycine.⁴⁰³

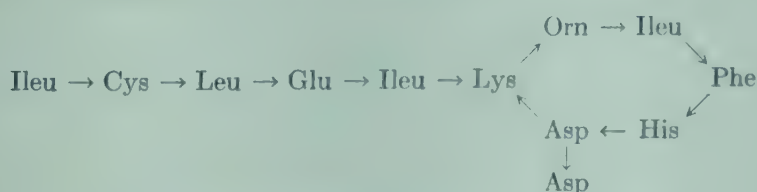
Preliminary analyses of the amino acid composition of acid hydrolysis products of bacitracin, using Moore and Stein starch columns, were obtained in 1948.³⁹³ More precise data on amino acid composition from hydrolysis of bacitracin A by hydrochloric acid, was achieved several years later by counter-current distribution studies, by ion exchange,⁴⁰⁸ and by paper chromatography.⁴⁰⁶

A quantitative picture of the amino acid residues in the molecule, however, was finally realized, from partial hydrolysis studies.⁴⁰⁹ Evidence showed that bacitracin A is composed of three *L*-isoleucine residues and one each of *L*-leucine, *L*-cysteine, *L*-histidine, *L*-lysine, *L*-aspartic acid, *D*-phenylalanine, *D*-ornithine, *D*-aspartic acid, and *D*-glutamic acid. In addition, hot acid also liberates one molecule of ammonia.^{407,408}

A vast amount of painstaking work in two laboratories,^{410,411,413} and to a lesser degree in a third,⁴¹⁶ was done in the fractionation of partial hydrolyzates, which combined countercurrent distribution with paper chromatography, paper electrophoresis, and ultimate analysis.⁴⁰⁷ The most substantial work was carried out by Hausmann, Weisiger, and Craig,⁴⁰⁹ but the independent efforts of the three groups supplied valuable evidence leading to the structure sequence of the amino acids in the bacitracin A molecule (CXXIV).^{406,407,410,413,414} In one laboratory,^{407,409} the partial hydrolyzates of bacitracin A were subjected immediately to countercurrent distribution. After 1450 transfers, Craig and his co-workers found that none of the maxima in the distribution diagram exhibited a homogeneous component; however, ten fractions forming the largest maxima con-

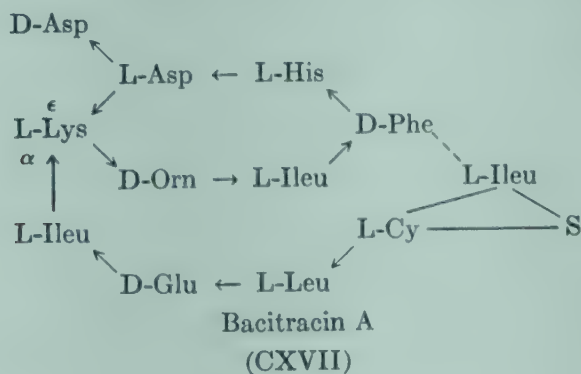
tributed largely to the constitutional determination. Portions of these fractions, each containing a related family of peptides, were subjected to two-dimensional paper chromatography and electrophoresis, further hydrolyzed, and analyzed for their amino acid composition by paper chromatography. The remaining portions of the ten fractions were reacted with fluorodinitrobenzene to form dinitrophenyl derivatives⁴¹⁴ and distributed in a suitable system. The patterns from these distributions were determined by ultraviolet absorption at a wave length of $350\text{ m}\mu$ ⁴⁰⁹ where the dinitrophenyl group possesses maximum absorption. These subfractions were hydrolyzed, and the dinitrophenyl-substituted and non-substituted amino acids identified and estimated after separation by countercurrent distribution.⁴¹⁵ On the other hand, Lockhart, Newton, and Abraham^{411,413} chose to study the amino sequence of bacitracin A by methods originally utilized by Sanger and Thompson⁴¹⁸ in their study of insulin.

When bacitracin A is fully substituted with fluoro-2,4-dinitrobenzene, three dinitrophenyl groups are attached. Hydrolysis and fractionation of the tridinitrophenyl derivative of bacitracin A revealed that the δ -amino group of the ornithine residue, and the NH of the imidazole ring of the histidine moiety in the antibiotic were free.^{403,406,412,417} The exact point of attachment of the third dinitrophenyl group was the subject of considerable difficulty and controversy. Because the dinitrophenyl derivative of isoleucine could be isolated only in poor yield,^{404,406} a great deal of experimental evidence and thoughtful consideration was put forth to establish the isoleucine as the N-terminal residue.^{403,410,413,421} The evidence from electrometric titration and dinitrophenyl derivatives were inconsistent with any conventional straight-chain peptide. Although linkages involving the lysine and aspartic acid residues have received much attention,^{420,409} partial hydrolysis studies showed that the lysine residue was joined at three positions indicating that it is a site of cross-linking of the chain (CXXIV).⁴⁰⁹ Further, one of the polypeptides showed that the carboxyl group of phenylalanine was coupled with the α -amino group of histidine to form a ring. Despite a number of ambiguities, the interpretation of these results led to the formulation indicated in CXXIV where \rightarrow signifies a C—N bond.^{410,406,411,412,415,420,421}



Sequence of Amino Acids in Bacitracin A
(CXXIV)

standing achievements of both American and British workers, give support to the structure of bacitracin A as represented by Abraham in CXXVII.⁴²² The dotted line in formula CXXVII depicts a bond which



has not been clearly interpreted. In discussing the nature of the sequences phenylalanyl-histidine and phenylalanine-isoleucyl-cysteine, Wrinch⁴⁹⁹ remarked that the failure to find direct evidence of multiple peptide grouping in partial hydrolysis products of a peptide does not prove that it is not present.

3. POLYMYXIN

In 1947 three independent groups of investigators, almost simultaneously, reported the discovery of antibiotic substances elaborated by various strains of *Bacillus polymyxa*. Benedict and Langlykke⁴²³ briefly described the isolation of strains of *B. polymyxa*, which produced an antibiotic under certain conditions of fermentation. Very soon afterwards, Stansly, Shephard, and White^{424,425} described the production and some properties of an antibiotic active against gram-negative organisms; they named it polymyxin. Further, they gave reasons for identifying the microorganism as a strain of *B. polymyxa*,⁴²⁵ and later described methods for purification.⁴²⁶ At almost the same time, Ainsworth, Brown, and Brownlee⁴²⁷ published a communication on the preparation and properties of aerosporin, an antibiotic produced by a bacterium called *B. aerosporus*, which had been isolated in 1946 from the soil of a market garden in Surrey. These British workers suggested the antibiotic-producing organisms were similar to *B. polymyxa*, and that aerosporin and polymyxin were closely related substances. Their work was amplified later by Brownlee and Bushby.^{428,206}

Further work showed that *B. polymyxa* is a species relatively common in soils and other habitats.^{429,430} Thus, other active substances from

strains of *B. polymyxa* were found which seemed to differ only slightly from polymyxin and aerosporin.^{431,432} In attempts to resolve common patterns of these antibiotics, particularly the question of the relationship of polymyxin and aerosporin, a conference was held by the New York Academy of Sciences in May 1948.⁴³³ Before these publications appeared in print, a number of problems had already been clarified.

The multiple nature of this group of closely related antibacterial substances was soon recognized by several investigators, particularly by Jones.^{434,435,436} By careful application of paper chromatography, Jones^{435,436} demonstrated that different strains of *B. polymyxa* produce different polymyxins. Further reinvestigation of the toxonomic derivation of *B. aerosporin* and *B. polymyxa* showed that the two microorganisms are identical. Aerosporin was renamed polymyxin A^{423,437,438,439} and polymyxin, suggested by Stavely, Shepherd, and White⁴²⁴ was designated

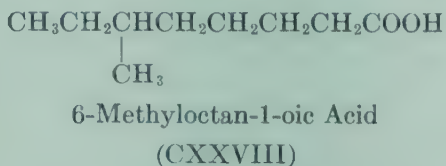
TABLE 2-4
COMPONENTS OF THE VARIOUS POLYMYXINS

Poly- myxin	(+)-6-Methyl- octanoic Acid	D-Leu- cine	L-Threo- nine	D-Serine	D-Phenyl- alanine	L- α - γ -Diamino- butyric Acid
A	+	+	+	—	—	+
B	+	+	+	—	+	+
C	+	—	+	—	+	+
D	+	+	+	+	—	+
E	+	+	+	—	—	+

polymyxin D. A comparative study showed that polymyxin D differed from polymyxin A in gross properties and, further, that the former contained serine in addition to a complete complement of components common to each antibiotic as shown in Table 2-4.⁴³¹

On the basis of amino acid composition, the British workers characterized and designated their discoveries as polymyxins A, B, C, E,^{437,435} and the American type as polymyxin D.⁴²⁴ These various polymyxins display selective but similar spectra of activity against gram-negative bacteria.^{162,439,441} Several salts of the antibiotics were prepared by base precipitants and acid azo-dyes.⁴⁴² However, Wilkinson⁴⁴³ crystallized the naphthalene β -sulfonate salt of polymyxins B and E, and converted them to polymyxin B hydrochloride and polymyxin E neutral sulfate. The purified polymyxins were all found to contain L- α , γ -diaminobutyric acid, L-threonine, and the same unknown optically active C₉H₁₈O₃ fatty acid.^{431,432,424,443} The α , γ -diaminobutyric acid was isolated by Catch and Jones⁴⁴⁴ who confirmed its structure by synthesis.^{444,440} This was the first

report of the occurrence of this acid in nature.⁴⁴⁴ Further, Wilkinson⁴⁴³ showed that the fatty acid present in all the polymyxins is an optically active acid, namely (+)-6-methyloctan-1-oic acid (CXXVIII).⁴⁴⁵

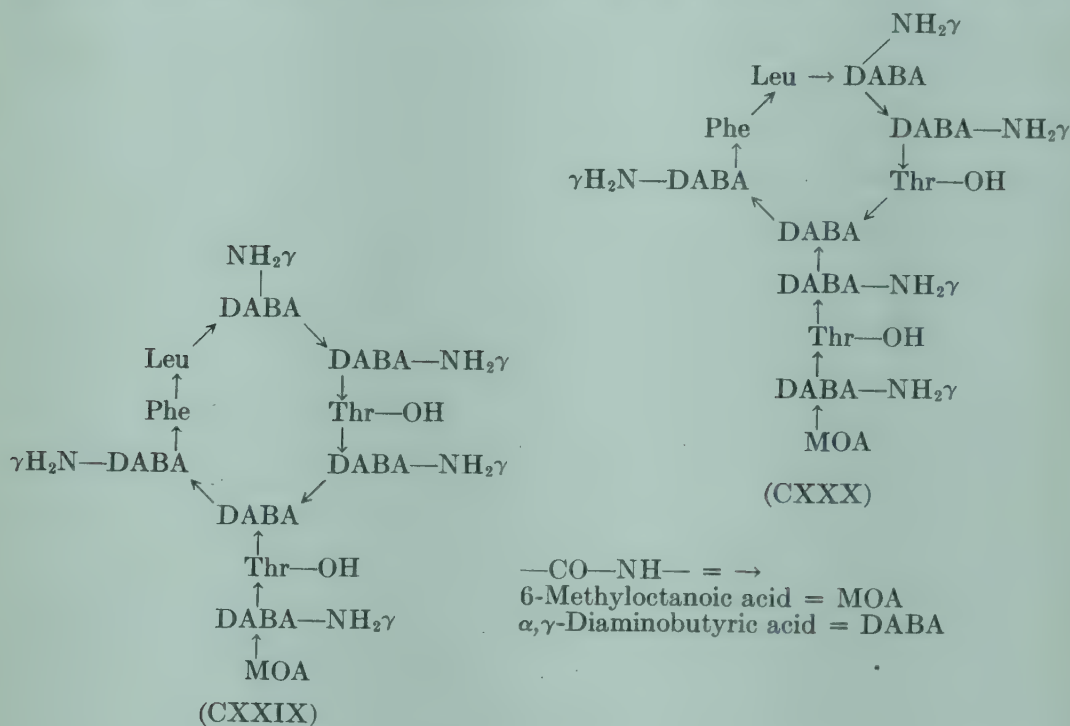


Single strains of *B. polymyxa* originally appeared to produce single antibiotics,⁴³⁶ but Regna, Solomons, Forscher, and Timreck⁴⁴⁶ showed by intensive purification that the strain which elaborates polymyxin B actually produces two slightly different peptides, which they called polymyxin B₁ and polymyxin B₂. The amino acid spectra of polymyxins B₁ and B₂ were found to be identical.⁴⁴⁶ Subsequent studies by Hausmann and Craig⁴⁴⁷ disclosed that the two peptides could be separated from crude polymyxin B by countercurrent distribution, and that B₁ and B₂ differ only in the nature of the fatty acid component present in each. Countercurrent distribution of the hydrolyzates gave fractions in which the presence of methyloctanoic acid (CXXVIII) was detected in B₁. But hydrolyzates of B₂ were found to contain an isooctanoic acid, C₈H₁₆O₂, whose structure has not yet been determined.⁴⁴⁷

The molecular weight of polymyxin B₁ was determined by the method of partial substitution, previously described, in which the purified antibiotic was reacted with fluoro-2,4-dinitrobenzene. The molecular weight was found to be $1150 \pm 10\%$.^{431,447} This value for the free base is in good agreement with the proposed molecular formula C₅₉H₉₉N₁₆O₁₄, based upon evidence for a free carboxyl group, and the assumption that all the components in the molecule are linked to form a cyclic polypeptide.^{431,447,448} Quantitative isolation studies by Hausmann and Craig,⁴⁴⁷ in which hydrolyzates of B₁ were subjected to countercurrent distribution, revealed that the molecule is composed of six moles of predominantly *L*-α,γ-diaminobutyric acid, two of *L*-threonine, one of *D*-phenylalanine, one of *L*-leucine and one of 6-methyloctanoic acid.

Laborious experiments to determine the amino acid sequence of polymyxin B₁ were carried out by Hausmann,⁴⁴⁸ in which dinitrophenyl-substituted polymyxin B₁ was hydrolyzed partially in acid, and the resulting peptide mixture was fractionated by means of multiple dialysis^{337,364} and countercurrent distribution in various systems. Fourteen substituted peptides were isolated in a state of purity indicated by the criteria of countercurrent distribution, paper chromatography, and paper electrophoresis. These peptides were substituted further with fluorodinitro-

benzene and studied, before and after hydrolysis, by countercurrent distribution and paper chromatography in order to determine the position of the amino acids. Of the fourteen fragments isolated and identified, only seven key peptides were necessary for structural deductions, although all the other sequences isolated were in agreement with the key peptides. The lack of a carboxyl group in intact polymyxin B₁ indicates that the constituents are arranged in a ring structure. Unfortunately the peptide bonds formed by the amino group of threonine are rather weak toward mineral acid; thus not a single threonine-peptide could be found where it was not N-terminal. For this reason it was necessary for Hausmann⁴⁴⁸ to propose two structures for polymyxin B₁, as shown in CXXIX and CXXX. The structure CXXIX consists of a ring of eight



Tentative Structures of Polymyxin B₁

amino acids and a tail ending with the methyloctanoic acid. The junction between ring and tail is made up of a completely-covered residue of diaminobutyric acid, an arrangement similar to the branch formed by lysine in the peptide chain of bacitracin A (CXXIV). Further work is required to determine the exact manner in which the completely covered diaminobutyric acid residue is linked, that is, whether the α- or the γ-amino group is involved in the ring formation.⁴⁴⁸ The only difference in CXXX is that one diaminobutyric acid residue is located in the tail instead of the ring. An unequivocal choice of one of the tentative struc-

tures CXXIX or CXXX must depend on experiments using sensitive cleavage methods, for example, enzymatic hydrolysis, which do not split the threonine bonds, but act rather on other bonds in the peptide.

4. NEOMYCIN

Neomycin was discovered by Waksman and Lechevalier.⁴⁴⁹ The antibiotic, elaborated by a strain of *Streptomyces fradiae*, was shown to be a heterogeneous mixture, and for this reason was designated the 'neomycin complex'.^{450,464} Neomycin is active against many gram-positive, gram-negative, and acid-fast bacteria,^{449,450} and has a biologic activity comparable to streptomycin.^{454,455} The microorganism *S. fradiae* also forms an antibiotic called fradycin, $C_{30}H_{34}N_4O_3$ ⁴⁵¹ possessing antifungal properties, but no activity against bacteria.⁴⁵² However, a strain of *S. albogriseolus* also produces the 'neomycin complex', but it is free of an antifungal component.⁴⁵³

The lack of well-defined methods for isolation and purification of single entities from these heterogeneous mixtures led to confusion during characterization of the antibiotic components comprising the 'neomycin complex'. After a series of unwieldy purification steps, Peck, Hoffhine, Gale, and Folkers⁴⁵⁶ were first to obtain a homogeneous, crystalline, biologically active substance from crude neomycin concentrates. The single component material was designated 'neomycin A'. Following this work and during the course of study of neomycin preparations, Regna and Murphy⁴⁵⁷ detected the presence of at least two biologically-active components in similar mixtures. On purification, one of these components exhibited biologic and clinical properties different from 'neomycin A'; therefore it was called neomycin B.⁴⁵⁷ Independently, Dutcher, Hosanky Donin, and Wintersteiner⁴⁵⁸ separated neomycin B and neomycin C as discrete components of the 'neomycin complex', and showed that these antibiotics were sharply differentiated from 'neomycin A'. Furthermore, they noted the similarity of their neomycin B to that reported by Regna and Murphy.⁴⁵⁷ Thus, at this stage of the work in 1951, investigators were led to believe that neomycins A, B, and C existed.

However, Wintersteiner and co-workers⁴⁵⁸ recovered from methanolysis of neomycins B and C [1] non-identical amorphous products which were named methyl neobiosaminides B and C, and [2] identical amorphous hydrochlorides for which an empirical formula of $C_9H_{19}N_3O_5 \cdot HCl$ was suggested. The composition of both fragments was corrected later, but the hydrolysis product [2] turned out to be the substance previously designated 'neomycin A' by Peck and associates.⁴⁵⁶ This experience was

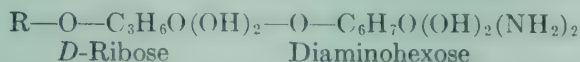
the first in which a degradation product of an antibiotic had demonstrated biologic activity. Further purification and crystallization of the amorphous compound [2] led Dutcher and Donin⁴⁵⁹ to revise their earlier molecular formula $C_9H_{19}N_3O_5$. The crystalline base they obtained proved to be identical with neamine, a substance which Leach and Teeters^{460,461} had isolated from acid hydrolyzates of purified neomycin B,⁴⁶² and which Peck and co-workers had obtained from the 'neomycin complex'. Later studies by Peck, Hoffhine, Gale, and Folkers⁴⁶³ showed that this base has the molecular formula $C_{12}H_{26}N_4O_6$. The name neamine was adopted for this fragment common to both neomycins B and C, and the term 'neomycin A' was abandoned. Kuehl, Bishop, and Folkers⁴⁶⁵ reported that continued hydrolysis of neamine in strong acids at elevated temperatures yields a fragment identified as the dihydrochloride of a $C_6H_{14}N_2O_3$ substance, the *meso* isomer of 1,3-diamino-4,5,6-trihydroxycyclohexane.

In attempts to characterize further the highly-purified isomeric neomycins B and C, Ford, Bergy, Brooks, Garrett, Alberti, Dyer, and Carter⁴⁶⁶ subjected these antibiotics to methanolysis under conditions used by Dutcher and co-workers.⁴⁵⁸ They confirmed the recovery of one mole of neamine hydrochloride, $C_{12}H_{26}N_4O_6 \cdot 4HCl$ per mole of neomycin B, neomycin C, and their respective methyl neobiosaminides more exactly formulated by Rinehart, Woo, Argoudelis and Giesbrecht⁴⁶⁷ as $C_{11}H_{21}N_2O_9(OCH_3)$: the purified form of compound [1] obtained originally by Dutcher, *et al.*⁴⁵⁸ Rinehart and co-workers⁴⁶⁷ using dilute hydrochloric acid, cleaved the anomeric α - and β -glycosides of methyl neobiosaminide C to α - and β -anomers $[[\alpha]_D^{25} + 113^\circ$, and $[\alpha]_D^{25} + 61^\circ$, respectively] of neobiosamine C ($[\alpha]_D^{25} + 104^\circ$), formulated as $C_{11}H_{22}N_2O_8$ (see Table 2-5). Their results argued for the formulation of neobiosamine C as a diaminohexosidopentose, which is cleaved with difficulty, and not as a pentosido-diaminohexose previously suggested.⁴⁵⁸ Similar reasoning was presented for structural relationships of neobiosamine B. Both α - and β -anomers $[[\alpha]_D^{25} + 13^\circ$, and $[\alpha]_D^{25} - 17^\circ$, respectively] were hydrolyzed to neobiosamine B of constant rotation ($[\alpha]_D^{25} + 33^\circ$), which was also formulated as $C_{11}H_{22}N_2O_8$, a diaminohexosidopentose.

The pentose moiety in both neobiosamines B and C was shown by Rinehart, Woo, and Argoudelis⁴⁶⁸ to be *D*-ribose. In their experiments N,N'-dibenzoylneobiosaminide C was hydrolyzed in dilute aqueous hydrochloric acid. Intensive purification of the mixture gave a neutral, salt-free carbohydrate fraction, which by color tests, papergrams and rotation was identified as *D*-ribose. The pentose derived from N,N'-dibenzoylneobiosaminide B gave a rotation corresponding to *D*-ribose and also formed an osazone identical with ribosazone. The formulas of

neobiosamines B and C (CXXXI), of methyl neobiosaminides B and C (CXXXII), and of neomycins B and C (CXXXIII) as indicated by Rinehart, Woo, and Argoudelis⁴⁶⁸ are given in Table 2-5. The difference between the isomeric antibiotics, it would appear, lies in the diaminohexose moieties. However, the structures of the diaminohexoses, the nature of their linkage to ribose, the position of ribose attachment to neamine, and

TABLE 2-5
FORMULAS OF NEOMYCINS B AND C



Neobiosamines B and C, R=H (CXXXI)

Methyl neobiosamines B and C, R=CH₃ (CXXXII)

Neomycins B and C, R=C₁₂H₂₅N₄O₅ (neamine) (CXXXIII)

the determination of the glycoside as either a pyranoside or a furanoside, are questions which can be answered only by further painstaking experiments.

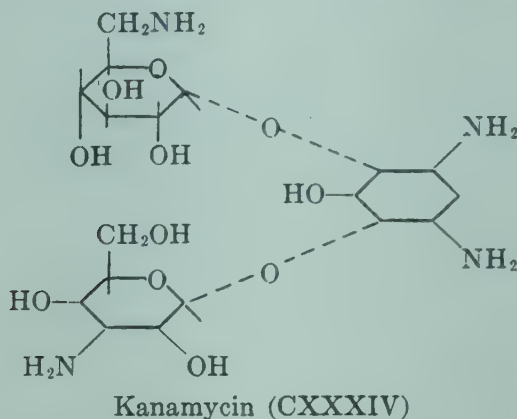
5. KANAMYCIN

Kanamycin has some of the antibacterial and chemical properties of neomycin and streptomycin. It is a water-soluble basic antibiotic with activity against Mycobacteria, gram-positive, and gram-negative organisms,⁵²² and contains, like neomycin, the 1,3-diamino-4,5,6-trihydroxycyclohexane moiety.⁵²³ The antibiotic was isolated from culture filtrates of *Streptomyces kanamyceticus*,^{522, 524} and subsequently characterized by Umezawa and co-workers.^{524, 525} Crude preparations reveal the presence of a second antibiotic, kanamycin B.^{523, 548} The infrared spectra of kanamycin and its B component are similar and typical of polyhydroxy-polyamino substances.

Crystalline kanamycin sulfate dried to constant weight analyzes for the molecular formula C₁₈H₃₆N₄O₁₁·H₂SO₄. The salt can be converted to kanamycin base $[\alpha]_D^{25} = +146$ (in 0.1 N H₂SO₄). The antibiotic gives Molisch, ninhydrin, and Elson-Morgan tests, and forms tetra-N-acetylkanamycin on reaction with acetic anhydride in methanol.^{523, 525}

Kanamycin is stable to treatment with methanolic hydrogen chloride under the same conditions which yield neamine from neomycin B and C. However, hydrolysis in 4N hydrochloric acid for 15 minutes degrades kanamycin into a mixture of products from which 1,3-diamino-4,5,6-trihydroxycyclohexane is isolated.^{465, 523, 546} In addition, the filtrate yields two amino sugars: 6-deoxy-6-amino-*D*-glucose (6-*D*-glucosamine),⁵⁴⁶ and 3-amino-3-deoxy-*D*-glucose (3-*D*-glucosamine).^{546, 547} Cron, Fardig,

Johnson, Schmitz, Whitehead, Hooper, and Lemieux⁵⁴⁷ suggest that the two amino sugar moieties are linked to the 4 and 6 position of the diaminotrihydroxycyclohexane moiety⁵²³ as indicated in the structure for kanamycin in CXXXIV. Although the diaminotrihydroxycyclohexane



moiety is a new *meso* form with all *trans* configuration, positions 4 and 6 are not stereochemically equivalent.⁵⁴⁷

Kanamycin B $[\alpha]_D^{25} + 135$ (in water) was isolated in pure form by countercurrent distribution of the salicylidene derivatives of the crude antibiotic mixture. Hydrolysis of N-acetylkanamycin B yields 1,3-diamino-4,5,6-trihydroxycyclohexane as the di-N-acetyl derivative and 3-*D*-glucosamine as the pentaacetate. The third moiety in the hydrolyzates of kanamycin B yields a ninhydrin-positive reducing test, but it is not 6-glucosamine present in kanamycin itself.⁵⁴⁸ Its identity should be revealed in subsequent investigations.

6. VIOMYCIN

Viomycin was isolated by Marsh, Mayer, Mull, Scholz, and Townley^{526, 543} in culture filtrates of *Streptomyces vinaceus* (*Actinomyces vinaceus*). At about the same time two other groups of investigators found that the antibiotic is produced also by closely related strains, namely *S. puniceus*⁵²⁷ and *S. floridae*.^{528, 529} The antimicrobial agent is effective particularly against both streptomycin-sensitive and streptomycin-resistant strains of tubercle bacilli.^{527, 528, 529, 530, 531} It is currently in use as an adjunct in the treatment of tuberculosis. Several attempts have been made to enhance the tolerance of viomycin, for example in combination with pantothenic acid.⁵³² The pantothenate salt of viomycin appears to reduce the calcium-binding effects of the basic *Streptomyces* antibiotics.⁵³³

During fermentation, *S. vinaceus* produces two and possibly three closely related antibiotics designated vinactin A, B, and C, which have not

been well-characterized.⁵³⁴ Viomycin is largely vinactin A. It is a strongly basic polypeptide with a molecular formula $C_{17-18}H_{31-35}N_9O_8$.^{535, 536} It is recovered during the isolation process as the hydrochloride,⁵²⁸ sulfate, oxalate,⁵²⁷ and picrate⁵³⁵ salts. Hydrated viomycin sulfate has $[\alpha]_D^{25} - 32^\circ$ (in water); it decomposes at about $280^\circ C$. It is highly soluble in water and virtually insoluble in most organic solvents.⁵²⁷ Van Slyke determinations indicate that the antibiotic possesses one primary amino group. Vinactin A and B appear to be similar peptides in which guanidino and creatinine groups have been reported. Preliminary studies suggest the presence of creatinine in vinactin C.⁵³⁵

Viomycin is relatively stable to strong acids; however, vigorous acid hydrolysis inactivates the antibiotic.⁵²⁷ Products of the acid hydrolysis^{535, 537} are carbon dioxide, ammonia, urea, *L*-serine, α, β -diaminopropionic acid, an unidentified guanidino component of approximate formula $C_5H_{10}N_3$ and β -lysine (β, δ -diamino-*n*-caproic acid: $NH_2 \cdot CH_2CH_2CH_2CH(NH_2)CH_2COOH$), identical with the amino acid isolated from streptothricin,⁵³⁷ streptolin,^{538, 539} and roseothricin.⁵⁴⁰

7. VANCONYCIN

The antibacterial agent, vancomycin, was isolated by McCormick, Stark, Pittenger and Pittenger³²¹ from strains of *Streptomyces orientalis* n.sp.,³²² a microorganism found in a soil in Indonesia.³²³ The antibiotic, primarily active against gram-positive and gram-negative cocci, has little effect on gram-negative bacilli. It has been shown to be predominantly bactericidal at low concentrations.³²⁴ In addition, vancomycin has a low order of toxicity in laboratory animals³²⁵ and in humans.^{326, 327, 328} It is not absorbed through the intestinal tract, and when administered intermuscularly or intravenously appears not to be metabolized to any great extent in the body.³²⁹

Vancomycin is a complex amphoteric material containing at least two active substances,⁵⁰⁴ which can be recovered from filtered broth by adsorption on ion exchange resin and removed by mixtures of glacial acetic acid, ethanol, and acetone. The antibiotic forms insoluble phosphomolybdic, phosphotungstic, lead, and mercury compounds. Crude material can be purified further by carbon adsorption and elution, and by precipitation as the picrate salt. The compound is converted to an amorphous white solid hydrochloride salt by precipitation in methanol containing dry hydrogen chloride.³²¹ Crystallization of vancomycin and its sulfate has been achieved by Higgins and co-workers.⁵⁰⁴ Non-crystalline material contains about 7% nitrogen and between 16–17% carbohydrate. Infra-red absorption spectra suggest the presence of hydroxyl or amino, amide,

and aromatic groups. Organic chlorine, glucose, aspartic acid, and an unidentified ninhydrin-positive substance were found to be constituents of vancomycin.⁵⁰⁴ Titration data indicate the average molecular weight of the antibiotic to be in the range 3200 to 3500 ± 200 .³²¹

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CHAPTER III

ANTIBIOTICS IN NUTRITION

BY T. D. LUCKEY

A. INTRODUCTION

1. ORIENTATION AND DISCOVERY OF GROWTH STIMULATORY EFFECT

A general concept of nutrition includes the processes by which material is obtained, stored, ingested, digested, absorbed, transported and utilized (excretion may sometimes be included) by a cell, a tissue, an organ, an organism or a population for energy, maintenance of molecular constituents, growth and reproduction. This concept provides the outlines of what we should like to know about antibiotics in nutrition. Some of these points are covered in subsequent chapters and much of the rest is known only for one or two specific antibiotics. Consequently few conclusions can be drawn from work in this field.

The subject of antibiotics in nutrition may be placed in proper perspective by a comparison of the state of our knowledge of vertebrate nutrition with a nutritional pattern which has been developed more fully, namely, microbial nutrition. After a given culture of microorganisms has been maintained on a crude medium, attempts are made to grow it on synthetic-type or artificial media, followed by work with a chemically defined medium. During this process, its qualitative nutrient requirements are learned, that is, we can state that the organism can use *these* energy sources, that it must have *these* minerals, that it requires *this* amino acid and *that* vitamin. After these qualitative nutritive requirements have been learned, the quantitative ones are often determined rather quickly. Organisms grow rapidly in media which contain all of their essential nutrients.

Frequently compounds are found which stimulate more rapid growth although they are apparently not dietary essentials, that is, the organism could live and reproduce without them. Thus serine, glycine, the Tweens and factor S stimulate growth in *Tetrahymena*,^{1,2,3} and thymine stimulates the growth of *Streptococcus lactis*.⁴ Fatty acids are stimulatory to many organisms.⁵ In a review of nutritive requirements of bacteria, Peterson and Peterson⁶ list many purines and pyrimidines as miscellaneous growth stimulants.

The mechanism of this stimulatory action may be different for each compound: it may be a molecule in a synthetic pathway, it may be easily converted to an essential compound, it may reduce surface tension or it may accelerate transfer through a cell membrane. Change in conditions can occur whereby these compounds become inactive.

We have learned from microbial nutrition that after essential nutritional elements were elucidated, non-essential growth stimulants were found. Therefore, since most of the essential nutrients for vertebrates⁷ have been elucidated, growth-stimulating compounds for them should also be known. Prominent among such early discoveries were growth promotion by inositol and p-aminobenzoic acid,⁸ by arabinose and glucuronic acid,⁹ and by ascorbic acid¹⁰ in chicks fed synthetic diets. Thus the unexpected finding of growth stimulation by antibiotics or sulfa-drugs by Moore *et al*¹¹ was actually within the accepted framework of nutritional patterns.

As microbiological nutrition studies may be regarded as the precocious brother, so the concept of the magic bullet of Ehrlich may be considered to be the mother of the growth stimulating effect of antibiotics. The lineage is direct and full. However, never during the course of the lock and key-antimetabolite conjugal development did anyone suspect that one of the charmed magic bullets might become an exciting boomerang. Could Ehrlich have predicted that a selective toxin would have a non-specific stimulatory action?

Parenthetically, it should be noted that when effective therapy was administered to infected birds, increased growth was both expected and found. This was exemplified by an increase in the performance of chicks infected with coccidia when they were treated with water containing phenylarsonic acid,¹² and chicks infested with round worms grew better when the disease was treated with quarternary nitrogen compounds.¹³ This apparently distinct theme was found to blend indistinguishably with that of antibiotics as growth stimulants during development of this field in the following 10 years.

The experiments which established the growth effect of antibiotics in animals over a period of years were guided by a scientific heritage which, although essential to continued progress, sometimes inhibits it by bringing about a traditional approach. The use of dietary sulfanamides produced growth depression in rats¹⁴ which apparently induced a biotin deficiency.¹⁵ Folic acid involvement must have been secondary, as it has since been shown that rats can synthesize folic acid (vitamin B₁₀) when biotin is provided.¹⁶ The use of dietary sulfasuxidine increased the folic acid requirement of chicks threefold.¹⁷ Such results were attributed by some

workers to an altered intestinal microflora induced by the drugs (although direct proof for such action is lacking).

Thus when antibiotics became available for non-medical experiments in 1945, it seemed reasonable to feed this new type of "drug or a combination of drugs that would completely inactivate all bacteria in the intestinal tract (which) would be highly desirable, since investigators would be provided with an essentially sterile animal, and vitamin requirements could be studied uncomplemented by 'intestinal vitamins' or toxic substances".¹¹ The experiment failed; feeding succinylsulfathiazole, streptomycin, streptothricin or the combination of streptomycin and succinylsulfathiazole failed to produce a sterile intestinal tract (although streptothricin did kill about 99 44/100% of the microorganisms before it killed the chicks). This feeding also failed to "lead to increased requirements for other vitamins or to new types of deficiencies." The quantitative bacteriological survey indicated that on a weight basis vitamins (folic acid) could be 10 times more active than either sulfa-drugs and/or antibiotics in changing the intestinal flora. Moreover the growth results were the opposite of those expected; the chicks fed sulfa drugs and/or streptomycin clearly gained 10-30% more weight than control chicks. What possible explanation could be given? The authors suggested "the inhibition of certain bacterial groups which might decrease the growth of the animal through either (1) the consumption and consequent immobilization of vitamins, or (2) the production of toxic compounds. However the possibility that (3) these agents are acting systemically cannot be overlooked."¹¹

Although this field of study has been very active in the past several years, adequate data (to be reviewed later) have yet to be accumulated to prove which of these or other possible modes of action is responsible for the increased growth rate observed in animals fed antibiotics.

The following year dried penicillin mycelium, fed in a practical ration, was found to give a growth boost to chicks,¹⁸ and in 1948 chlortetracycline in the diet was also found to increase the growth rate of chicks.¹⁹ The effect of antibiotics (and arsonilic acids²⁰) on growth was confirmed in 1949 when the beneficial effect of feeding antibiotic fermentation residues in plant protein diets was found to be greater than could be attributed to the vitamin B₁₂ content of the residues.²¹ The active factor was found to be the residual antibiotic,²² and in 1950 growth stimulation by dietary antibiotics was reported in chicks and pigs by many investigators.^{23,24,25,26} The power of the calliope in the antibiotic band wagon increased spectacularly during the next few years while poultry, pigs and patents danced its tune. Geese, pheasants, chicks, turkeys, pigs, mink, calves, rats, mice and men responded to the stimulus of antibiotics.

Although much good work remains to be done in the field, the amount

of work reported on antibiotics in nutrition during the past year is but a sprout compared to the forest of work done in the preceding five years. The present is an opportune time to review the work to date. The literature has been surveyed through 1957.*

B. ANTIBIOTICS IN FEEDS

Poultry grow twice as fast on one-half as much feed today as they did 20 years ago. Swine, calf and cattle feeding has improved tremendously. Better nutrition, improved management and purposeful breeding are part of the answer. But the effective use of antibiotics was also a major contribution. The addition of antibiotics to feeds is quantitative, not qualitative, in nature. Antibiotics are by their very definition of natural origin and they probably existed at least sporadically in *trace* amounts in feeds and diets prior to the present decade.

1. FORTIFICATION OF FEEDS WITH ANTIBIOTICS

Commercial production of several medications to be added to water or feeds began early in the third decade of the twentieth century.²⁷ Tobacco dust was used for roundworm infestations in poultry, colloidal iodine for worms, coccidiosis and blackhead, mineral mixtures for a variety of purposes, sodium fluoride for worms in swine, and powdered sulfur for coccidiosis in poultry. Many local concoctions were used. In the fourth decade of this century, medicated feeds and fortified feeds (mineral and vitamin) were used extensively on a large scale. Fortified and medicated feeds are now accepted as a major part of a feeding program, particularly in areas of concentrated production.

The feeding of antibiotics presents no new mechanical problems to the feed manufacturer; for he had previously mixed small quantities of minerals and vitamins into his fortified feeds. The vitamins are unstable to heat, oxidation and storage; antibiotics present similar problems. The new problem is that these are medicated feeds. The manufacturer may assume responsibility for mixing antibiotics, and other drugs, in a prophylactic fortification program, but he needs to work much closer with the veterinarian when he takes the apparently short step from prophylactic feeding to therapeutic feeding. Every man is responsible for disease prevention, but the cure of animal disease is the chosen profession of the veterinarian. Closer cooperation of these two groups with the farmer as well as with government and drug manufacturer is needed.

* This task has been aided and eased considerably by the availability of the excellent series of annotated bibliographies available from the Veterinary Research Department of Merck and Company, Inc.

Antibiotic feeds were regarded as drugs by the Food and Drug Administration,²⁸ but in 1951 they were exempt from the certification required for drugs on the ground that they were to be used solely to promote the rate of growth. When it became evident that a given antibiotic feed is effective in the prevention or treatment of specific infectious diseases, certain preparations containing prescribed amounts of antibiotics were also made exempt from certification (under section 146.21, January 1956).

TABLE 3-1
DRUGS CURRENTLY USED IN ANIMAL FEEDS*

New Drugs	Certifiable Antibiotics
arsenosobenze	bacitracin (also zinc salt and methylene disalicylate)
carbarsome	chloramphenicol
dienestrol diacetate	chlortetracycline
diethylstilbestrol	dihydrostreptomycin
hygromycin	penicillin
nicarbazin	procaine penicillin
2-acetyl-amino-5-nitrothiazole	streptomycin
2,4-diamino-5 (p-chlorophenyl)6-ethylpyrimidine	tetracycline
2,2'-dihydroxy-3,3', 5,5'-tetrachlorodiphenyl sulfide (bithionol) and	
4,6-diamino-1-(4 methylmercaptophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5 triazine hydrochloride (methiotriazamine)	
*Not New Drugs	
acetyl (p-nitrophenyl) sulfanilamide	4-nitrophenyl-arsonic acid
aminonitrothiazole	iodinated casein
arsanilic acid	menadione sodium bisulfite
cadmium anthranilate	oxytetracycline
cadmium oxide	para aminobenzoic acid (Na and K salt)
di-N-butyltin dilaurate	pepsin
dinitrophenylsulfonylethylenediamine	phenothiazine
dried rumen organisms	piperazine
erythromycin thiocyanate	piperazine hexahydrate
furazolidone	piperazine monohydrochloride and dihydrochloride
2,2'-dihydroxy-5,5'-dichlorodiphenyl methane	piperazine phosphate monohydrate
3-nitro-4-hydroxyphenyl arsonic acid	piperazine sulfate
nicotine	sodium arsanilate
nitrofurazone	sodium fluoride
nitrophenide	sodium propionate
	sulfaquinolaxine
	trimethylhexadecyl ammonium stearate
	trimethyloctadecyl ammonium stearate

* These or other substances are drugs or even new drugs under specific conditions of use and representation. List complete through February, 1958.

of Regulations in the Federal Food, Drug and Cosmetic Act). A list of drugs presently used in feeds is given in Table 3-1. Each of these is regulated by the Food and Drug Administration. Many of them can be added to feeds at stated levels for a specified disease or condition with no predistribution testing of each batch or certification of the preparations. Thus the government and the drug manufacturers are necessarily cooperating to prevent any government inspection program from becoming unwieldy. The administration allows certain combinations to be used. In all cases they must be conspicuously labelled. Some require adequate directions and warnings for use. A glance at the variety of drugs which may be in medicated feeds shows how this field has grown in these few years. Almost all mixed feeds sold today have antibiotics in them.

The chief antibiotics used by themselves in commercial feedstuffs today are chlortetracycline, procaine penicillin and oxytetracycline. Bacitracin and streptomycin are used, usually in conjunction with one of the other compounds. A combination of 2 or more antibiotics is usually not better than a good one fed singly.^{29,30} There is apparently enough experimental evidence³¹ to cause companies to include 2 antibiotics in some feeds. Tyrothricin, gramicidin, and neomycin have been reported to give a growth response.³² Oleandomycin and erythromycin also promote growth in³³ chicks and poults. As is explained later in this chapter, inactivated antibiotics and certain non-antibiotics give similar responses.

The question of which antibiotic is best is most controversial. References could be cited showing that there is no essential difference between the important antibiotics used,³⁴ and other references could be cited indicating any one of the "big five" was better than another. Suffice it to say that one can set up an experiment (or quote an experiment) to show that one antibiotic is better than another.

In some work on antibiotic administration, it is found that addition of the antibiotic to water or salt blocks instead of to feed has been used on occasion. Subcutaneous implantation of antibiotics has not proved to be equivalent to oral administration,^{35,36,37} although sometimes bacitracin pellets give growth stimulation to pigs.³⁸

Antibiotics are also added to most feeds for laboratory animals. It is a real problem for feed manufacturers to obtain ingredients for mixing antibiotic-free diets for certain laboratory purposes. In experiments where milk is to be fed to laboratory animals, antibiotics (about 20 ppm) are sometimes added to keep the milk from fermenting during the day.

Removal of antibiotics from the diet usually causes a loss of any accelerated growth advantage, and may cause a decreased growth or actual weight loss. Ruminants are more sensitive to antibiotic discontinuity than are animals with simple stomachs.³⁹

2. COMBINATIONS: ANTIBIOTICS PLUS OTHER GROWTH STIMULANTS

Combinations of growth stimulants are being tried today. Evidently farm feeding will become a tremendously complex problem with the choice of which and how much mineral, vitamin, antibiotic, hormone and tranquilizer should be added to poultry, swine and cattle feeds. Experiment in this field have hardly begun to explore the possible combinations or permutations where both time and level are important.

There is one indication that arsonic acid gives additive effects with low level feeding but not with high levels of antibiotic.⁴⁰ A conflicting report indicates that the combination of antibiotics with arsanilic acid does not give additive effects,^{41,42} and may show less activity than either separately at the concentrations usually fed. Feeding antibiotics and female sex hormones together shows activity that may give an additive effect on weight increase in steers.^{44,45,46,47,48,51} Other reports indicate no additive effect.^{43,49,50,52} The effects of antibiotic plus CuSO_4 feeding can be additive in pigs⁵³ and chicks.⁵⁴ The combination of antibiotic or hormone feeding with tranquilizer implantation may give added daily increase in weight.⁵⁵ No reports have been seen of experiments in which more than two different types of growth stimulants were used at one time. It is obviously premature to draw conclusions from this interesting and active field of investigation. An equally provoking question to be left for future study is whether antibiotics allow lowered vitamin supplementation of practical feeds. Indirect evidence would indicate the possibility, but practical experiment must determine the feasibility of such penny-pinching.

3. CONCENTRATION OF ANTIBIOTIC IN FEEDS

The variety of methods used to express the concentration of pure antibiotics in feeds should cause few problems because most of them are equivalent. The usage in this chapter standardizes on ppm (parts per million), except in citation of references. Other abbreviations, equivalent to parts per million, include: γ/gm (gamma per gram); $\mu\text{g}/\text{gm}$ (micrograms per gram); mg/kg (milligrams per kilogram); $\text{gm}/1,000 \text{ kg}$ (gram per thousand kilograms); $\text{mg}/2.2 \text{ lb} \cong \text{mg}/2 \text{ lb} \cong \text{gm}/2,200 \text{ lb} \cong \text{gm}/\text{ton}$. The only other term commonly used is the mixed term; mg/lb , and it may well cause trouble. The quantities involved in this term (which should not be used at all!) are about two parts per million. Per cent is sometimes used; since it means parts per 100 parts, one per cent is 10,000-fold greater concentration than 1 ppm.

These expressions are used to express the amount of antibiotic in the

complete ration and in concentrates as presented to the animal. To avoid confusion, their use in concentrates intended for feed manufacturers and in impure concentrates or crude fermentation residues is not discussed.

In the early 1950's, the antibiotic levels used in feeds varied from 2-5 ppm. for very low levels (growth responses have been reported using less than 1 ppm) to 5-15 ppm. Since then the tendency has been to increase the level of antibiotics in commercial feeds. Levels as high as 100, 200, and even 300 ppm are now frequently reported in the literature. These concentrations constitute prophylactic feeding, while therapeutic feeding includes these levels and extend up to 2,000 ppm.

The more general terms of "low level feeding" and "high level feeding" are often used to show whether the level fed is high enough to give bacteriostatic concentration in tissues. High level feeding is used when an infectious disease is expected or in progress. Low level feeding was used more generally in the early part of the decade; however, the level used seems to be gradually going higher in commercial feeds as time passes. Christenson⁵⁶ and others⁵⁷ have judged high level feeding to involve concentrations of 50 ppm or more. However, Gordon⁵⁸ showed that levels of 16 ppm in the feed led to bacteriostatic levels in tissues. Luckey⁵⁹ has given a good reason to consider levels below 25 ppm as "low level feeding." Germfree chicks grew faster when levels of antibiotics below 25 ppm diet were fed, and gave no response to high levels.

Despite the variables of molecular weight, potentiators, salt forms or derivatives, and the inherent potentiality of the several antibiotics, a need for a practicable dividing line between "high," and "low" level feeding remains. From a consideration of the work above, an arbitrary compromise of 0.1 to 25 ppm is suggested as being "low level."

Feed formulation problems handled at farm feed trials will tell management which are the most practical antibiotics (laboratory experiments will not necessarily give the proper data). Such work allows the economic factors to be evaluated. The best antibiotics may be too expensive, particularly if royalties must be paid to another company. The stability of the compound may be adequate for field tests but entirely inadequate to give the needed "sack-life." The crystalline form or diluent may be changed to increase shelf-life stability. The antibiotic may function better as an insoluble, stable derivative (this is the advantage of procaine penicillin). Highly purified preparations may be more unstable than less pure preparations. Or, as in the case of penicillin, the pure product may be much more stable. The amount of moisture present in the feed may have considerable effect on the stability of one preparation and have little effect on another. The stability in a pelleted food, a mash, a salt mixture or in water must be anticipated. Continuous aerosol application in

broiler plants has not been compared to continuous feeding. Sometimes liquid concentrates are sprayed onto food mixtures, and sometimes liquid (water or glycerol) is added prior to pelleting. The effect of different levels in the gut and on the animal remains one of the big decisions to be made by the feed manufacturer.

The level of antibiotic to be added must be carefully considered. While high level feeding may be best under one animal management system, the cheaper low level feeding would be adequate for another. A given amount of antibiotic put in a pre-mix package may be most convenient for one feed manufacturer, but may present quite a problem when one recalls that some firms produce less than 1,000 tons of feed annually and others approach 4 million tons annually. It is indeed a problem to mix 1, 10 or 100 grams of material evenly throughout each ton of feed. 1/100,000th of the total must be added accurately and mixed thoroughly. The answer seems to be to add a diluent to the pure antibiotic. It is relatively easy to mix 1 part of antibiotic in 100 parts of diluent. (One pound of antibiotic in diluent per ton is sometimes found convenient.) The feed manufacturer gives a manufacturer's concentrate. The feed manufacturer likes an inexpensive, dilute mixture, while the chemical manufacturer finds a high potency concentrate easier to mix and cheaper to ship. The diluent must be nonhygroscopic in behavior and clean in appearance, and have the proper pH, particle size and density (so it will not settle out after mixing). It should also have some nutritional value, be free from detrimental impurities and be acceptable to the trade, while still maintaining enough character to be identified easily as the desired product. One of the chief characteristics, which has not been well considered, is the palatability of the antibiotic, or the combination of antibiotic and diluent, to the animal to be fed. The taste, the color, and particularly the odor are important. The combination should flow properly in mixing machines with a minimum of dustiness, caking or production of static charge. The diluent should be low in cost so that it can be used in both high and low potency concentrates.

The feed control laboratory must devise methods to extract the antibiotic for testing potency. This information, together with a study of stability during storage, shipping and sales determines the overage required (how much extra antibiotic must be added to give the required amount after the feed has been mixed, packaged, shipped, stored and finally fed).

These are the every-day problems of the industry. They multiply in products with two or more additives. They have evidently been solved to the extent that the use of fortified feeds is the most effective, simple and economical method for mass treatment of flocks or herds of animals.

C. DIGESTION AND METABOLISM

1. DIGESTION AND ABSORPTION

It must be remembered that this chapter deals with antibiotics presented to the animal every day in its feed over a sustained period of one or more months. The supply to the intestinal tract, blood and tissues is continually being renewed as the animal eats. The frequency of supply may have an important bearing upon the results obtained. In experiments wherein the antibiotic is injected, the frequency of injection may be the major difference between success and failure to obtain a response. The results from feeding may depend upon the same variable.

Results obtained with different species may reflect their eating habits. A general correlation is seen between the frequency of food intake during the day and the effectiveness of the antibiotic growth promotion. If the antibiotic is fed twice a day with a feed concentrate at roughly 12 hour intervals (e.g., at 6 a.m. and 6 p.m. when a farmer does his chores) the tissue levels would fluctuate much more than if the animal ate 4-5 times per day when the food is presented *ad libitum*. Minks, foxes, cats, dogs and fish finish their *one* meal a day within a ten minute period. The level of antibiotic in the intestine, blood and tissues would be expected to rise to a high level within one hour and gradually decrease after 2-3 hours to a low level, long before the next meal twenty-four hours later. Tissue levels would fluctuate much as if the antibiotics had been injected once a day. This method would be a poor way to elicit a reaction in the tissues, which might depend upon a relatively constant cellular level. When the antibiotic is fed in a concentrate to farm animals, it is usually consumed twice a day, and foals, horses, cattle, some lambs, sheep, and swine usually receive the antibiotic in this way. Rats eat only two or three times during a 24-hour period (about 1:00 a.m., 9:00 a.m. and sometimes about 4:00 p.m.). Young animals also are often fed 3-4 times each day. Institutionalized children eat 3 meals a day, in an arrangement that leaves a long period from about 7 p.m. to 7 a.m., when the tissue concentrations would be expected to decrease considerably. When calves, lambs or pigs are fed *ad libitum*, they usually eat 4-5 times during the daylight hours. While the length of day varies relatively little in Florida, the length of night (sunset to sunrise) in our northern states varies from 8 hours in the summer to 15 hours in the winter. Therefore during each 24-hour period, these animals eat about twice as rapidly, and fast almost twice as long in winter, as they do in summer. Consequently the variations in blood and tissue level of an antibiotic from the food must be greater in winter. Could this affect the results from feeding antibiotic? To answer this question more readily, consider the problem that it presents in Norway

and Sweden—at Trondheim there is no night for two months in summer and only 7 hours of sun in the dark of winter.

Chicks and guinea pigs represent the other end of the “frequency of eating” spectrum. They eat at frequent intervals, often hourly, during daylight. With the exception of the cecum, food passes rapidly through the intestinal tract in both species. This nibbling should keep a relatively constant level of antibiotic in the intestine, blood and tissues during the waking hours. Since, as Aristotle observed, nature gives to one part what she takes from another, this difference may be nature’s way of balancing the variations in the absorptive processes in the different species.

TABLE 3-2
ABSORPTION AND EXCRETION OF ANTIBIOTICS⁶⁰

Antibiotic	Absorption	Excretion
penicillin*	rapid	urine
streptomycin*	poor (2%)	feces, urine, ² bile
tyrothricin	none ¹	none, digested
bacitracin*	none ¹	urine ²
polymyxin	none	urine ²
erythromycin	easy	urine and feces
carbomycin	easy	1% in urine
oxytetracycline*	easy	urine
chlortetracycline*	easy	urine
tetracycline	good	urine
chloramphenicol	rapid	urine ³ and bile

* Commonly used in foods.

¹ A small amount is absorbed when large quantities are given.

² Excretion route after injection.

³ Less than 10% excreted unchanged.

The absorption of the antibiotics used singly in feedstuffs is quite rapid (chlortetracycline, oxytetracycline and penicillin), while absorption is slow for those antibiotics (streptomycin and bacitracin) which are used commercially in combination with one of the other three. The ease and rate of absorption of these and other antibiotics are presented in Table 3-2. Little systematic work has been reported on the metabolism of these antibiotics in the body.

Penicillin may be broken down by the acid of the stomach and by penicillinase in the digestive tract (from bacteria harbored therein). For this reason more stable and less soluble derivatives, such as procaine penicillin, are more effective. Even with them, very little antibiotic reaches the lower gut. (Other antibiotics are more stable than penicillin.) Dilution by digestive juices, destruction and absorption constantly decreases

the concentration of the antibiotic in the gastrointestinal tract, while excretion via bile, reabsorption of water in the bowel, and the phenomenon of bioincrustation¹⁶ (the absorption of relatively greater proportions of carbohydrate, protein and fat) balance this loss. The concentration in the feces or lower tract may thus be higher than that in the food.⁶¹

2. TISSUE STORAGE AND BLOOD LEVELS

The quantity of antibiotic found in tissues of animals fed these drugs is important from the theoretical as well as the practical viewpoint. The subject is reviewed in Chapter 8 from the public health standpoint, and in chapter 7 from the point of view of food preservation. In the present chapter the interest in tissue levels is primarily to help in determining whether there can be an antibiotic action directly on the tissues which might cause the growth acceleration.

Welch⁶² has reviewed the absorption and excretion of oxytetracycline, chlortetracycline and chloramphenicol in humans. Blood serum levels of 1-3 γ /gm were maintained by oral doses of 250 mg. every six hours. Doubling or quadrupling the dose gave very slight increase in blood serum levels of the tetracyclines, while that in the level of chloramphenicol was even less. The total urinary excretion of chlortetracycline was much less than that of the other two drugs when all were given as a single oral dose of 1 or 2 gm. When the same quantity was given at six hour intervals, less chloramphenicol was excreted in the urine. Thus, oxytetracycline consistently shows greater urinary excretion. More oxytetracycline is also excreted via feces when fed as a single oral dose of 0.5, 1.0 or 2.0 gm. Much of the chloramphenicol excreted is inactive bacteriologically. Presumably it is inactivated by bacterial enzymes in the intestinal tract. Tissue studies in rabbits given 0.5 gm of the drug by stomach tube showed low levels of all three drugs present in blood, brain, skin, liver, kidney and lungs. Chloramphenicol levels were high in the spleen, bile, heart and urine, while the tetracyclines were low in these tissues. The gut content was high; 500-700 ppm in all cases. Chlorotetracycline continued to be excreted 3-4 days after administration to patients was discontinued.⁶³ Someone should determine levels in depot fat after antibiotic administration. The levels of antibiotic in rumen fluid of calves disappeared rapidly after oral administration of chlorotetracycline.⁶⁴

Blood levels of tetracycline were about twice as high after feeding a single dose of tetracycline phosphate as they were when tetracycline hydrochloride⁶⁵ was fed. In neither case were they as high as was reported for the above compounds. Bile levels of tetracycline were rather con-

stant during the period following oral administration. Glucosamine appears to potentiate the activity of tetracycline:⁶⁶ higher blood levels of the drug are obtained when it is fed with glucosamine than when fed with citrate or phosphates. It should be recognized that natural feeds would have different amounts of potentiators, as well as other compounds which might decrease the absorption of the antibiotic.

Gordon⁵⁸ reports that when the level of antibiotic in the diet is 16 ppm or more, detectable levels (bacteriostatic) are found in blood and other biofluids. When 0.5 mg of antibiotic per kg of body weight was injected intramuscularly, the concentration found in the intestine was equivalent to feeding 3 ppm. The methods of Taylor⁵⁸ are apparently much more sensitive than that used by workers reported below.

TABLE 3-3
QUANTITY OF ANTIBIOTIC IN TISSUES OF
CHICKS FED CHLORTETRACYCLINE

Amount fed, ppm	Amount present, ppm		
	Blood serum	Liver	Lean meat
10	0.00	0.00	0.00
50	0.019	0.018	0.009
100	0.012	0.043	0.023
200	0.051	0.085	0.035

Durbin *et al*⁶⁷ found chlortetracycline in blood, liver and muscle of chicks fed 50, 100, 200 or 2,000 ppm. Vavich *et al*⁶⁸ report that no antibiotic was found in chicken flesh when chlortetracycline was fed at 100 ppm, and variable quantities were found when 1,000–2,000 ppm were fed. When chicks were fed chlortetracycline for 6 weeks, the antibiotic was found in the serum, liver and lean meat.⁶⁷ The quantities reported are given in Table 3-3. In calves, livers and kidneys contained measurable amounts of chlortetracycline when 400 mg were injected weekly, while in calves fed 50–90 mg daily, only traces were found.⁶⁹ No antibiotic was found in the spleen, thymus, pituitary and muscle in either group. None was found in the rumen of antibiotic-injected calves.

Luther *et al*⁷⁰ report very little or no detectable antibiotic (oxytetracycline) present in muscle meat of swine or poultry fed 50 gm per ton of food, appreciable quantities were found when 200 gm of antibiotic was fed per ton of food.

These data establish within rough limits the amounts of antibiotic which must be fed before bacteristic levels are detected in tissues. Levels required for stimulation of bacterial cells are usually of a lower order of

magnitude. Feeding high levels of antibiotic for only a few days provides enough in tissues that the carcass is as well preserved as by dipping after sacrifice.⁷¹

The soluble antibiotics are excreted primarily via urine. The insoluble ones are excreted in feces. Some, as penicillin, chloramphenicol, and presumably the polypeptide antibiotics, are largely inactivated before they get through the intestine. Penicillin and chlortetracycline are reported not to cross the placental tissues of the pig.⁷²

D. EFFECT ON VERTEBRATES

Apparently the effect of antibiotics upon vertebrates is tempered almost as much by the condition of the vertebrate being fed as they are by the level of antibiotic in the feed. The environment may be equally important. With the exception of the situation wherein antibiotics are fed to heavily infected animals, a typical animal from a group fed antibiotics would be very difficult to distinguish from a typical animal fed the same diet without antibiotics. The effects of antibiotics are subtle changes which are best determined by objective examination to determine such characteristics as weight, nutritive requirements, morphology, tissue composition, food efficiency, or cost per unit gain.

In the following literature survey, reports giving no growth differences were admittedly less valuable than those wherein a difference was seen. Many reports were not included simply because they duplicated other work which was better performed or reported.

1. SURVIVAL

More frequent survival of small or weak animals is one of the most striking results from feeding antibiotics. In this situation, as in the growth response, the sooner the animals are given antibiotics after birth the better are the results. Increased survival of animals infected or infested with harmful microorganisms is one of the chief reasons for the present-day "high level" feeding of antibiotics. The cost of treatment is negligible where a number of animals may be saved.

Increased survival of premature infants was reported by Robinson⁷³ and Snelling and Johnson.⁷⁴ Embryonic mortality was decreased by the addition of antibiotics to the diet of laying hens.⁷⁵ Survival of pig embryos was not affected.⁷⁶ Survival of chicks was increased by the addition of chlortetracycline to diets partially deficient in vitamin B₁₂,⁷⁷ and mortality in turkey poults was decreased when penicillin was given soon after hatch.⁷⁸ The decrease in early mortality and the lowered incidence or severity of disease in heavily populated poultry areas are im-

portant factors which make antibiotic feeding an economically sound practice.

2. GENERAL APPEARANCE

The general appearance of animals fed antibiotics does not differ on an individual basis from that of control animals which received no antibiotics. A comparison of a typical animal from the group which received antibiotics with a typical animal fed the same diet with no antibiotic shows no characteristic distinction, except where disease clearly exists. The best animals in each group are almost identical in body size, general conformation, alertness, bearing, eye clarity, appearance of excreta, skin condition, and quantity and quality of fur or feathers. A comparison of the poorest 10–20% of animals in each group usually reveals the differences which make it worth while to feed antibiotics to a billion animals in this country every year. Antibiotics help the runts, the weaker animals, those more likely to fail under the rigors of their newly-found environment. Among such animals, those fed antibiotics can usually be distinguished easily; they are larger, more alert and thrifty in appearance; they have fewer problems of scours, eye discharge, colds, diarrhea, and loss of skin integrity (particularly around the umbilicus of ruminants). Improved feathering may be seen⁷⁹ in poultry. It is primarily the performance of these small or poor animals which gives the overall advantage to those animals fed antibiotics, resulting in improved uniformity of the entire group. It is fortunate that the present methods of nutrition are sensitive enough to determine differences that represent the delicate balance between the animal and its environment. These are the types of changes which have led to the concept of “sub-clinical” disease.

3. GROWTH

The most exciting effect of antibiotics upon vertebrates is that of increasing the growth rates. In general the feeding of antibiotics has little or no effect on normal animals reared under excellent management and fed a complete diet, but as mentioned above, it does increase the growth rates of runts, weak animals, animals in poor environmental conditions, infested or infected animals, or animals fed a diet which is inadequate in some respect. This increase means that some cells grow larger in the same time period and others have increased mitotic rates. (The rate of tumor growth in antibiotic-fed animals should be studied.)

a. Humans

Jukes⁸⁰ reviews the effect of antibiotics upon the growth rate of human subjects. Some workers report improved growth of premature infants

when fed antibiotics, others do not. Several different workers report increased growth of children when antibiotics are administered. Often the children being studied were not in the best condition. Children receiving sub-optimal calories or protein were the most responsive.⁸¹ Growth in young adult males (Great Lakes Naval Training Station) was also improved by penicillin or chlortetracycline. With the possible exception of premature infants, it is difficult to visualize practical reasons for continuous antibiotic feeding of humans.

b. Chicks

Since more work has been done with chicks than other animals, more effects have been reported for this species. Under certain conditions as little as 0.2–0.5 ppm of penicillin is active in promoting the growth rate of

TABLE 3-4

COMPARISON OF DIFFERENT ANTIBIOTICS ON THE GROWTH OF CHICKS TO 8 WEEKS OF AGE

Antibiotic	All plant protein	2% Fish meal in the plant protein
None	100	100
Streptomycin	107	102
Oxytetracycline	111	105
Chlortetracycline	111	107
Bacitracin	112	111
Procaine Penicillin	116	110

* 9 ppm of antibiotic in the feed.

chicks above that of controls fed no antibiotic.⁸² The data given in Table 3-4 illustrate the variety of antibiotics which were found to be effective in this way in an early experiment. In this and other early studies,⁸³ 2 gm. procaine penicillin per ton of diet was as active as higher levels. The values are relative weights based upon the weight of birds fed no antibiotic being set arbitrarily at 100. This value is the growth index.

The growth index is defined as:

$$GI = \frac{\text{No. } \sigma \frac{\text{Av. Wgt. } \sigma \text{ fed drugs}}{\text{Av. Wgt. } \sigma \text{ fed no drugs}} + \text{No. } \varphi \frac{\text{Av. Wgt. } \varphi \text{ fed drugs}}{\text{Av. Wgt. } \varphi \text{ fed no drugs}}}{\text{Total No. of birds}} \times 100$$

There appears to be an inverse ratio between the rate of growth on the basal diet and the response obtained with antibiotics. Waibel⁸⁴ reported

data accumulated over a three-year period (Fig. 3-1a) showing that the response obtained with antibiotics is best when the growth of the controls is relatively less than it is at other times. The effectiveness of dietary antibiotics has been shown to decrease over the past few years under laboratory and farm conditions.^{33,85} Part of this change may possibly be attributed to the better diets used, to the increase in proportion of energy to protein in the diets, and possibly to a change in the character of the incipient flora resulting from the continued use of antibiotics. A similar curve could be drawn to illustrate that the effectiveness of antibiotics increases (particularly at high level feeding) with the disease level (Fig.

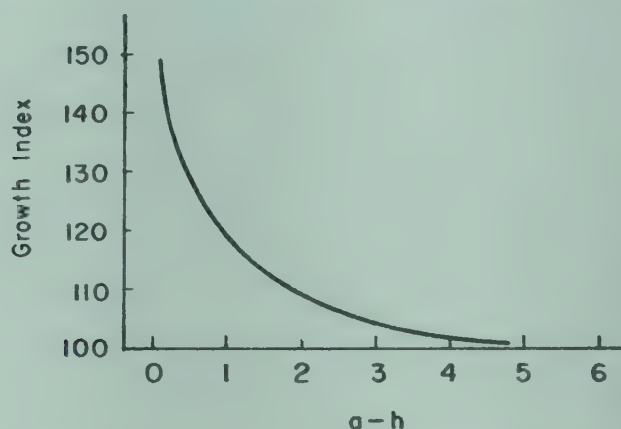


FIGURE 3-1. Factors in the response of animals to dietary antibiotics. The ordinate in the above graph is, as shown, the growth index, and the abscissa may be any of the following factors: a) Growth on basal diet (0-2 = poor; 4-6 = good). b) Disease level (0-2 = high; 4-6 = low). c) Relative size at birth (0-2 = runt; 4-6 = normal). d) Completeness of the diet (0-2 = poor; 4-6 = good). e) Cleanliness of environment (0-2 = runt; 4-6 = clear). f) Temperature stress (0-2 = large stress; 4-6 = no stress). g) General management (0-2 = poor; 4-6 = good). h) Age antibiotic feeding started (weeks).

3-1b). The same type of curve could be drawn to illustrate the marked tendency of antibiotics to stimulate the growth of runts to a greater extent than that of average-size or large young animals (Fig. 3-1c). Since these runts are not genetic runts, their growth potential is as great as that of their brothers and sisters. That is, most runts are primarily multiparous-birth animals, which may come from the extreme end of the uterine horn; one may speculate that they were semi-starved *in utero* and are weak because of a less efficient blood supply.

A similar curve would be obtained if the abscissa were changed to the age when the animals were first fed, or to the adequacy of the diet (amount of the protein or vitamins) (Fig. 3-1d) or to cleanliness of the premises

(Fig. 3-1e). In each case the results mirror the conditions under which the antibiotics are fed; antibiotics help the animal under stress (Figs. 3-1f or 3-1g). In this respect antibiotics are biological stabilizers. Since the drastic change in environment of an animal coming into this world from either the uterus or egg must put great stress on a large proportion of individuals, the closer to this period that antibiotics are given, the greater the value of the antibiotics (Fig. 3-1h). Birth usually brings a break in the growth curve; for many animals there is an actual weight loss after birth. No work has been reported wherein the antibiotic was provided during this period of great stress. Beginning the feeding of antibiotics at the age of one month produces little growth increase in birds⁸⁶ and calves.⁸⁷

Withdrawal of antibiotics from the feed results in a cessation of the accelerated growth rate⁸⁸ which indicates the material must be fed continuously. Few reports have appeared on experiments with discontinuous feeding. Such work would be helpful in delineating the mode of action. Injections once a day or feeding to animals which eat only once or twice a day may give poor results due to the variation of the concentration of antibiotic in cells, tissues, biofluids or intestine.

There are indications that high level feeding will promote growth rate when the low level will not; this is evidently true for animals reared in new quarters as well as those in dirty quarters.⁸⁹

c. Turkeys and Other Birds

Turkey poults also respond to antibiotics.^{24,90,91,92} The response in poults appears to be more consistent than that obtained in chicks as long as young birds are used. Growth response to antibiotics fed to both chicks and poults is greater with birds reared on litter than those reared on wire.⁹²

Other species of birds which have been reported to grow faster in the presence of antibiotics are goslings,^{93,94} quail,⁹⁵ and pheasants.^{79,96} Ducks respond to antibiotics^{97,98,99} but not as readily as other birds.^{100,101}

d. Pigs

The response of swine to dietary antibiotics¹⁰² reviewed by Cunha,¹⁰³ and Braude¹⁰⁴ shows about 15% faster growth rate, a fact which allows earlier marketing. The increased growth seen when well balanced rations are used is less than that obtained with poor rations; the response varies with the type of food as well as the quality. The response is due partly to increased appetite and efficiency of food utilization; it is greatest

under conditions of stress, such as poor sanitation. The greater the disease level in the animals being fed, the greater the quantity of antibiotic needed and the greater the growth difference seen between those animals fed antibiotics and the controls.¹⁰⁵ Pigs with scours and runt pigs responded more than others, which make the overall appearance and weight of a herd of the animals more uniform. Pigs fed restricted diets gained more when given antibiotics than those fed no antibiotic.¹⁰⁶ Food efficiency was improved.

Data have also been reviewed showing that the response to antibiotics is greater in young pigs than in older pigs and is most useful in creep feeding and synthetic milks. The antibiotics must be given continuously or the growth rate will be less than maximum. Combinations of antibiotics generally seem to be no better than any good one which is active for the species. However, this question is still to be resolved. Usually 10 to 25 ppm of oxytetracycline or chlortetracycline, which are more effective than penicillin, bacitracin or streptomycin for swine, are sufficient in experimental trials. Farm conditions may involve more stress for the animal, and thus higher levels are sometimes useful. The response obtained with pigs on pasture was sometimes less than that obtained in dry lot feeding.¹⁰⁷ The response varies depending upon the feed used and the condition of the pigs. Healthy pigs may not respond to antibiotics because "the disease level is too low".¹⁰⁸ High level feeding (50 to 1,000 ppm) is useful in the prevention or treatment of (growth retardation due to) swine dysentery, scours, enteritis and non-specific digestive disorders.

Many antibiotics are of little benefit in practical swine feeding. These include chloramphenicol, neomycin, subtilin, rimocidin, and polymyxin, although on occasion these have been found to be somewhat effective.^{109,110,111} Tetracycline has given increased feed efficiency but no increased growth¹¹² rate.

Injection of procaine penicillin G was found to have some activity.^{110,113} Discontinuous feeding of antibiotics does not result in optimum gains.^{114,115} Subcutaneous implantation of antibiotics does not stimulate growth^{116,117,118,119,120} unless it is done the first 2 days of life as in one report with bacitracin.¹²¹ The effect of antibiotics was greater when lactose was fed (50% level) than when glucose, sucrose, dextrin or cornstarch were fed.¹²² Since lactose depressed the growth rate, this result may be a part of the stress effect.

e. Ruminants

Calves were benefited by feeding antibiotics for 2-3 months.^{123,124,125,126,127,128} Injected antibiotics were sometimes active^{129,130} and sometimes

lot.¹³¹ Growth rate was increased, and incidence and severity of scours was reduced, feed consumption and efficiency were increased and general condition and appearance may have been improved. The financially important reason for feeding antibiotics to calves is the decrease in scours and digestive disturbances which reduces the mortality. This would be the only justification for feeding antibiotics to breeding stock which should not be made to grow too fast or to get too fat. Apparently about 40 ppm is the level to be used.¹³² Less benefit is expected for artificial milk fed calves¹³³ or more mature animals^{134,135} unless stress such as feedlot infections, shipping exposure, or bad weather are encountered. Increased growth or food efficiency is not always seen in ruminants, and the growth improvement noted may be too insignificant to warrant feeding antibiotics to mature animals.

Lambs also were sometimes benefited from antibiotic feeding^{103,136} whether fed by pellets, or non-pelleted food in the feed lot or in creep feeding. Loss of appetite and decreased crude fiber digestability were also reported.¹³⁷ It sometimes takes ruminants more than two weeks to show beneficial results. This period of acclimatization was sometimes not easy for the animal.¹³⁸

f. Horses

Foals fed chlortetracycline grew at a faster rate than control colts when given 200 mg per day from 13 weeks until weaning.^{139,140} When 100 mg. per day was given prior to 13 weeks no significant growth increase was seen. Other workers found no benefit from feeding penicillin or chloramphenicol to adult horses¹⁴¹ or foals.¹⁴²

g. Dogs

The increase in weight¹⁴³ and somewhat better food utilization in antibiotic fed puppies was due primarily to increased fat.^{144,145} There is then no reason to feed antibiotics to pups in most parts of the civilized world.

h. Fur Animals

Antibiotic feeding was not beneficial for breeding minks but growth of young was stimulated¹⁴⁶ and increased pelt sizes were obtained.¹⁴⁷ Fox kits were not helped when fed antibiotics with an all vegetable diet.¹⁴⁸ These data from effects on fur bearing animals were confirmed by work in Norway¹⁴⁹—antibiotics were found to be helpful only in cases of chronic diarrhea. Chlortetracycline may be harmful to chinchillas, while neo-

mycin and bacitracin appeared to help clear up the syndrome¹⁵⁰ caused by feeding chlortetracycline.

i. Other Animals

Rabbits usually do not^{151,152,153} respond to dietary antibiotics, depending upon conditions as yet undetermined. No overall benefits were found in guinea pigs fed low levels of chlortetracycline.¹⁵⁴ In most studies antibiotics appear to be quite toxic to guinea pigs.

Chlortetracycline, penicillin, streptomycin and chloramphenicol stimulated rat growth.¹⁵⁵ Chlortetracycline increased the growth of weanling mice,¹⁵⁶ particularly when fed soybean or cottonseed meal diets.¹⁵⁷ Increased growth was seen in kittens fed antibiotics.¹⁵⁸ Oxytetracycline, chloramphenicol, penicillin or chlortetracycline did not affect the growth of brown trout fingerlings.^{159,160} While little work has been done on species other than the usual laboratory or farm animals, chloramphenicol or chlortetracycline were found to increase the pupal weight and body growth in silkworms.^{161,162}

Growth stimulation in plants and microorganisms is reviewed under the mode of action in this chapter.

It is important to note that in this chapter the effect of antibiotics upon only the *rate of growth* has been the consideration. The weight of the animals at maturity is not affected. Therefore as the animals approach their mature weight, the weights of the controls (no antibiotics) gradually approach those of the animals fed antibiotics.

4. MORPHOLOGY

Although no change is seen in general body conformity, the carcasses of antibiotic-fed animals usually grade high. No change in slaughter carcass character of steers¹⁶³ was observed. While many organs were found to be heavier in calves fed antibiotic, the calves were heavier, and no difference was seen when computation was made on the basis of per cent of body weight.¹⁶⁴ Differences to be noted were differences in organs on a proportional basis (usually given as per unit body weight).

a. Liver

Increased liver weight was seen in rats¹⁶⁵ fed chlortetracycline. Chickens showed no change in liver weight when fed procaine penicillin¹⁶⁶ or sodium penicillin.¹⁶⁷ However Burgess *et al*¹⁶⁸ report a decrease in liver weight per Kg body weight in antibiotic fed chicks. Braude *et al*¹⁶⁹ reported no difference of liver, kidney, spleen weight or gut length in pigs fed chlortetracycline.

b. Endocrine Glands

Oxytetracycline gave increased adrenal weight in rats¹⁷⁰ with decreased adrenal chloesterol. The drug also stimulated adrenal cortex activity, with a reduced eosinophil count and a rise in free steroid sex hormones excreted in the urine. The general picture displayed resembled the activity of ACTH.¹⁶⁵ Chicks showed no change in adrenal weight.

Gordon found antibiotics stimulated the increased size of the thymus gland (in one group at 125 days) and number of thymocytes in conventional animals in which no growth stimulation was noted.¹⁷¹ No change was seen in the male sex organs of rats fed oxytetracycline.

Some workers report increased thyroid weight in antibiotic fed animals,^{170,172,173,174} and others indicate that no change occurs.^{175,176,177} Evidently uncontrolled variables exist which produce such conflicting results. (This problem has an important bearing upon future investigations and courses of action.) No change was seen in spleen or spleen lymphocytes in chicks fed procaine penicillin (which did not show any growth increase). Spleen weight was reduced in pigs fed chlortetracycline.¹⁷⁸

c. Intestinal Wall

The intestine assumes a position of great importance in every view of the role of antibiotics in nutrition. It encloses and provides the milieu in which food is solubilized and microorganisms flourish. The cells lining the gastro-intestinal tract are more specialized and probably more sensitive to change than most bacteria and yeasts with which they are in competition for nutrients. The mucosal cells are responsible for secreting a large volume of "intestinal juice" into the tract, and they absorb enough nutrients for the host to grow without themselves being digested by the various enzymes present. The absorptive area is 10 times greater than the body surface area of the animal (about 9 square meters or 10 square yards in man).

The cells of the intestinal wall are enzymatically among the most capable cells in the body. They are "eating from the same board" as the bacteria within the lumen. They are subjected to the same changes in intestinal environment. There is little reason to suspect that a biologically-active compound such as an antibiotic would have profound effect upon the microbial cells within the intestine, and yet have no effect upon the cells which are partially in the environment. Human nutrition is not merely a matter of feeding bacteria, the man is there also. One speaks of the microorganisms which are small enough to have great absorptive powers, and overlook the fact the intestine is made for absorption. The cells lining the tract have the primary function of testing, and then of

absorbing or rejecting the many species of molecules within the intestine. Changes in the body metabolism may well be reflected in secretions of these cells, and thus affect the intestinal flora.

Gordon *et al* have confirmed their finding that the feeding of antibiotics decreases the weight of the gastro-intestinal tract.¹⁷¹ This occurred even when no growth response was noted. They reviewed this field, and reported no change in the size of the cecum in conventional animals fed procaine penicillin, but the size of the cecum did decrease when chicks were fed oxytetracycline.¹⁶⁶ The wall of the intestinal tract was lighter in the experimental birds. These changes could not be correlated with changes in the microbial flora. No histological changes were noted by Coates *et al*.¹⁷⁹

Germfree birds showed no significant change in the digestive system or the lymphopoietic system when given penicillin or oxytetracycline.¹⁷¹ The cecal tonsil and lymphocytes are decreased in conventional birds fed procaine penicillin, but not when they are fed oxytetracycline. There is a tendency for the tonsil and lymphocytes to increase when germfree birds are fed procaine penicillin. Decreased tonus was seen in isolated loops,¹⁸⁰ the average diameter is smaller, villi were shorter and the tunica propria layer was thinner¹⁸¹ in birds fed antibiotics. Penicillin is reported to decrease the pH¹⁸³ and surface tension¹⁸³ of intestinal contents in chicks.

It would seem reasonable that the changes seen in conventional birds fed antibiotics which tend to make them resemble the morphologically germfree birds are mostly microbially induced changes. The antibiotic tends to restore these conditioned tissues to their original condition prior to the microbial stimulus.

Similar results are reported for pigs.^{80,169} It should be pointed out that copper sulfate which also stimulates growth in swine, tends to make the intestinal tract shorter and lighter in weight.¹⁷⁸ In rats, copper sulfate, penicillamine or copper arsanilate did not give a positive growth response, although each of these substances reduced the intestinal weight significantly.¹⁸⁴

5. INTESTINAL FLORA

A review of the intestinal microflora changes which have been reported is summarized in Table 3-5. It is apparent that conflicting data are absent only when there is no more than one report on the topic. A vague pattern is seen since the majority of workers report that feeding antibiotics increased the number of *E. coli* organisms in the tract, and that yeasts appear in relatively large numbers. Beyond this there seems to be no clear-cut trend. The lack of agreement is disheartening. Evidently a new approach or standardization is needed in this field. Results from

studies on the rumen microflora and the effect of antibiotics on rumen digestion is in a similar state of confusion.⁸⁰ Workers seem to agree that dietary antibiotics do not help cellulose digestion. Reviewers^{185,186,187,188} indicate that the total microbial population is generally not reduced, disease producing strains do not generally emerge (except possibly in chlortetracycline-fed guinea pigs)^{189,190} and no agreement is seen in papers relating to specific changes. Antibiotics also affect the higher parasites¹⁹¹ as well as bacteria and fungal population. It was already noted that other biologically active compounds such as folic acid¹¹ are more active,

TABLE 3-5

Antibiotic	Species	<i>E. coli</i>	Anaerobes	Aerobes	Enterococci	Lactobacilli
Chlortetracycline	pig	+	+	+	+	+
	chicks	-,0	0	-,0,0	0+	---,+,0
	ducks	---	-	-	-	-
	rats	+			+	+
Oxytetracycline	chicks				+	
	ducks	+	+	+	-	-
	rats	+	-	-	-	-
Penicillin	pigs	+				
	chicks	-,0,+,+,+	0,-,-,-	0,-,-	+,0,---	0,-,-
	ducks	+	+	+	-	-
	rats	+	-		0	0
Streptomycin	chicks	-	-		-	0
	ducks	+	-	+	+	0
	rats	-				

Note: Compiled primarily from references 80 and 171.

0 = no change: + = increase: and - = decrease. One symbol is given for each report on that subject.

on a weight basis, in changing the intestinal flora than are some of the antibiotics. Presumably changes in body fluids and tissues would also act in the intestine to change the flora.

In high level antibiotic feeding, resistant strains of bacteria were occasionally noted^{192,193} but certainly not as much as was expected.

6. CHEMICAL COMPOSITION

a. Carcass

It was quickly evident that dietary antibiotics increased growth and food efficiency in pigs and poultry at very little extra cost—more meat in less time at less cost. Must something be wrong? Old timers recalled the “salting” of steers in past decades. Therefore critical examination of

the carcass composition was of great importance. Reports of increased water consumption by antibiotic-fed animals added to the importance of analysis of carcass composition and judgment of carcass quality. When all the data are examined, it is seen that antibiotics are a sound investment for the feed dollar.

In restricted feeding tests, antibiotic fed pigs¹⁰⁶ showed a slight increase in the dressing percentage. This effect was greater in pigs fed grain as the chief part of the feed. Antibiotics fed under conditions of restricted feeding gave increased growth rates, with no effect of thickness of back fat, on weight of leaf fat, or thickness of streak, or on scoring of lean meat, and no increase in body length or amount of meat (on a percentage basis) in the pigs. No change was seen in percentage of dry matter, percentage of crude fat or of fat-free dry matter in the eye, muscle, or tenderloin of the pig. Similar data were obtained by others^{194,195,196} with non-restricted feeding.

With *ad libitum* feeding (practiced in most parts of the world) one problem arises. Hogs bred for good quality lean bacon apparently produce a greater quantity of fat bacon when fed antibiotics.¹⁹⁷ In the American breeds the data is less clear.¹⁹⁸ Some workers report increased total fat and back fat. The back fat was thicker¹⁹⁹ and had a lower iodine number, with less protein and less moisture,²⁰⁰ in the antibiotic fed animals. Others^{201,202} report no change in back fat thickness, weight of leaf fat, per cent of lean meat, carcass depth, carcass length, tissue moisture or specific gravity. The pattern that appears is that increased fat is produced when low protein diets are used. Rats fed meat from pigs receiving antibiotics and rats fed meat from pigs fed no antibiotics grew at the same rate.²⁰³ The nitrogen balance study on the rats indicated greater efficiency in the rats fed meat from pigs receiving no antibiotics. When compared to controls, swine fed chlortetracycline showed no significant difference in percentage of water, or lipid or of protein in the carcass, shoulder or ham composites.²⁰⁴

No change was seen in the carcass composition in turkeys²⁰⁵ from the viewpoint of crude protein, crude fat, ash or water. Turkeys fed penicillin may have improved carcass quality due to increased subcutaneous fat.²⁰⁶ Chicks may have more total fat, although fat does not account for all the growth increase.²⁰⁹ Mice fed antibiotics grew faster, with more tissue protein and neutral fat, but less liver fat (when on a high fat diet).²⁰⁸

b. Blood

There is general agreement that the following components for blood are not changed as a result of antibiotic feeding: hematocrit,^{156,164,168,209,210}

red blood cell count,^{156,164,171,209,210} hemoglobin,^{156,168,210,211} serum protein,^{156,211} urea nitrogen,²¹² creatinine,¹⁶⁸ fatty acids,²¹³ cholesterol,¹⁶⁸ plasma inorganic phosphorus, vitamin C,^{156,211} tocopherols,²¹¹ or B vitamins,^{213,214} such as riboflavin,^{156,211} biotin,¹⁶⁶ and vitamin B₁₂.¹⁶⁶

Serum alkaline phosphatase²¹¹ and plasma non-protein nitrogen may be reduced, the latter only during the period of accelerated growth.^{168,212} Plasma phospholipid,²¹⁵ glucose^{168,204,215,216} and whole blood folic acid¹⁶⁶ concentrations are greater in antibiotic-fed animals than in controls. Blood lipid,^{164,209} ascorbic acid,^{156,167,210} carotenoids,^{167,210} and plasma calcium²¹⁷ are found to be normal or somewhat above normal. Blood glucose rises higher after glucose administration in calves fed antibiotic.^{218,219}

Germfree birds fed antibiotics showed no change in dry weight, ash or B-vitamin content of the blood.¹⁶⁶

Chloramphenicol, oxytetracycline and chlortetracycline were found to stimulate antibody titer when fed at 0.1% level to rats and mice for a short time. When fed longer than two weeks the antibiotic appeared to interfere with antibody production.²²⁰ Penicillin increased total white cell counts and circulating lymphocytes,^{220a} while the gamma globulin fraction of blood is reduced.^{220b}

c. Adrenal Gland

Adrenal content of ascorbic acid and cholesterol were unchanged in chicks fed antibiotics.

d. Muscle

The thiamin, riboflavin and niacin content of ham did not change greatly when antibiotics were added to the diet of swine.²²¹ Nor were differences found for acid soluble phosphorus, nucleic acid phosphorus, ammoniacal nitrogen, nucleic acid nitrogen or total solids, although protein, lipid and p-proteins were increased somewhat.²²² Rabbits fed antibiotics had more muscle and liver glycogen than control rabbits.²²³

e. Liver

The liver of chicks fed 50 ppm oxytetracycline showed no change in dry weight, niacin, biotin, or vitamin B₁₂ content. The liver ash content was high and the folic acid content was low.²²⁴ In the liver of penicillin fed chicks (some of which showed increased growth and some did not), there was greater dry weight, but no change in protein, lipid or cholesterol.¹⁶⁸ When streptomycin was fed, no change was seen in liver dry weight, ash, folic acid or vitamin B₁₂.²²⁴

Conflicting reports continue: feeding streptomycin increased the vitamin B₁₂ content liver^{225,226} and feeding penicillin did not.^{226,227} Antibiotics apparently increase the vitamin A content of liver^{169,167,228,229} in chicks, while the results with pigs and rats are controversial.^{80,198,226} No change was seen in vitamin A or carotene content of the liver of steers fed chlortetracycline.³³⁰ Rat liver decreased in content of niacin, folic acid, pyridoxine and vitamin B₁₂ when antibiotics were fed with incomplete diets.²³¹ Penicillin supplementation had no effect upon riboflavin content of liver.²³² No change in liver fat (or liver function) was noted from the administration of chlortetracycline to rats, dogs or humans.²³³

f. Intestinal Contents

The B-vitamin (thiamin and riboflavin) content of the rumen of calves was not decreased by feeding 44 ppm chlortetracycline.²³⁴ Amino acid, riboflavin and niacin content of the rumen may be decreased in fistulated steers.

In chicks fed oxytetracycline, the cecal contents were similar to those of control chicks in dry weight and niacin; the ash content tended to be low; biotin and vitamin B₁₂ content were high, and the folic acid values were very high (over 700 γ /gm).²²⁴ In chicks fed streptomycin, no change was seen in the ash, folic acid and vitamin B₁₂ concentration of cecal contents, while the dry weight, niacin and biotin appeared to be higher than in control birds. Bone ash is increased when antibiotics (penicillin) are fed to chicks in a diet low in vitamin D.²³⁶

7. METABOLISM

Tissue composition changes and decreased nitrogen, vitamin, or mineral requirements in animals fed antibiotics may be interpreted as being, or reflecting, a changed metabolism. The following more direct changes in metabolism are reported:

Body temperatures are lower in calves¹⁶⁴ and sheep²³⁷ fed antibiotics, while no change was seen in lambs.²³⁸ Increased urinary ketosteroids were seen in guinea pigs fed antibiotics.²²³ More acetate (C¹⁴ study) was incorporated into hepatic fatty acids in rats fed chlortetracycline than in control rats.²³⁹ The intestinal wall of chicks can convert carotene into vitamin A more effectively when the chicks are fed antibiotics.^{228,240,241} The activity of enzymes in the pancreas²⁴² and intestine²⁴³ is increased. *In vitro*²⁴⁴ and *in vivo*²⁴⁵ experiments indicate a decrease in amines produced by intestinal microorganisms in the presence of antibiotic.

Other metabolic changes were seen in or reflected in studies on food efficiency and reproduction in animals fed antibiotics.

8. EFFECT ON APPETITE

Animals and birds generally are found to eat more food when fed antibiotics. The appetite factor has been referred to in many papers. Tomlin *et al*^{246,247} and others²⁴⁸ report palatability tests in which pigs preferred food in which chlortetracycline, penicillin V or erythromycin were present (40 ppm). They ate little diet with no antibiotic in free choice experiments and obviously dislike diet containing erythromycin. Swine seem to find oxytetracycline more tasty than chlortetracycline.²⁴⁹ This work should be repeated with other animals for evaluation as one mode of action of antibiotics in growth stimulation.

9. FOOD EFFICIENCY

Food and water consumption are usually found to be increased (on a per animal per day basis). Corn treated with fungicide was found to decrease food consumption of steers,²⁵⁰ although no weight change was seen. Food efficiency of broiler chicks was increased by 2 ppm procaine penicillin.⁸³ Similar results were found for turkey poults.²⁹ However, others suggest²⁵¹ that increased food intake in the first few days of life account for the growth increase. Chlortetracycline is reported to decrease the time of a passage of food through the intestinal tract of chicks.²⁵² Food efficiency is increased in pigs¹⁰³ and rats by several antibiotics whether given orally or parenterally.²⁵³ Milk utilization is greater in calves fed antibiotics.²⁵⁴

10. NUTRITIVE REQUIREMENTS

Antibiotics in the diet appear to reduce the requirements for vitamins, minerals and protein. This effect may be distinct from the growth stimulation on complete diets. It may well be a part of the increased food efficiency seen in antibiotic-fed animals. These effects must be considered separately until more is known about them—correlations cannot be made with our present lack of knowledge. Reviews^{80,198} of the subject may be consulted for greater details and interpretations of the literature to 1955.

a. Minerals

Chicks^{254a,255} fed antibiotics appear to drink more water. Urine output also increased in pigs^{201,256} and lambs.^{237,238} Slinger *et al*²⁵⁷ suggests that increased water consumption is not required to obtain a growth response in poults with restricted water intake. They suggested that a sparing action exists.

High calcium in diets may be required for a good antibiotic effect.²⁵³ Calcium appears to be utilized better²⁵⁹ as judged by increased uptake of Ca^{260} by increased bone ash in chicks,²⁶¹ increased calcium serum levels and shell-breaking strength of eggs from hens fed penicillin.²⁶² Blood levels from a single dose of Ca^{45} were lower in antibiotic-fed animals and bone growth was greater.²⁶³

It has been suggested that an apparent reduced requirement for manganese^{254a,264} in the presence of penicillin or chlortetracycline was mediated through the intestinal flora,^{254a} possibly by lowering the pH.²⁶⁵

b. Energy, Fat and Carbohydrate

Chlortetracycline was reported to increase the digestability of lipid in steers,²⁶⁶ to have no effect in dairy calves^{267,268} and to decrease it in sheep.²³⁷ Penicillin increases the efficiency of the utilization of energy.²⁴³ Blood glucose increases higher in antibiotic or surfactant-fed calves than in control calves when given glucose orally.²¹⁸ Baumann²⁶⁹ reports experiments with isolated sections of intestinal walls from rats and chicks in which those fed antibiotics allowed faster diffusion of xylose through them than did the walls from animals fed no antibiotics.

Consideration of these somewhat conflicting data with the evidence that the temperature of calves and sheep is reduced, and food utilization generally is increased, suggests the possibility that the energy requirement is reduced in animals fed antibiotics.

c. Crude Fiber Digestion

Crude fiber digestion studies done *in vivo* are considered with no attempt to include *in vitro* studies. Chlortetracycline in steers (100 mg/day) decreased the digestibility of crude fiber, but at 13 mg/100 lb body weight it had no effect.²⁷⁰ Penicillin had no effect.²⁷¹ No change was seen in digestion of roughage by calves^{267,268} or sheep^{237,272} fed chlortetracycline. Others report a decreased digestibility.²⁷³ This problem is of particular importance to those interested in the preparation of sheep and cattle for market.

d. Nitrogen Compounds

Antibiotics are generally found to improve the digestability of proteins^{267,268,274,275} and the absorption of nitrogen,²⁷⁶ to increase the efficiency of utilization of protein²⁷⁸ (particularly when somewhat low levels are fed), and essentially to reduce the total protein requirement.^{277,278} No effect on urea utilization,²⁷⁸ as well as impaired digestibility (in ruminants)^{237,271} or utilization of protein has also been reported. Evidence suggests that

more is involved than simply increased nitrogen retention,²⁸⁰ although this is quite dramatic in experiments where the combination of 2% protein with copper sulfate and penicillin was equivalent to 12% protein.²⁸¹

Ferrando *et al*²⁸² suggested that antibiotics increase intestinal permeability as judged by the increased nitrogen absorption observed in isolated intestinal loops to which chlortetracycline had been added.²⁸⁴ C¹⁴ L-lysine absorption was faster in chicks fed antibiotics.²⁸³ Chance *et al*²⁸⁴ indicated amino acid removal from the rumen of fistulated steers is increased after chlortetracycline is fed.

In vivo experiments with rats showed that lysine, leucine, methionine and cystine absorption was improved.²⁸⁵ This action may be at the expense of a decreased deposition of fat in rats.²⁸⁶ Rats fed a low casein diet (low in tryptophane) responded to a variety of antibiotics (at 0.01%) as well as to tryptophane.²⁸⁷ Rats fed streptomycin showed increase nitrogen retention, while other antibiotics did not have this effect, which therefore does not explain the general protein sparing action of antibiotics.²⁸⁰

e. Vitamin Requirements

The large amount of work done on the effect of antibiotics on the vitamin requirement of animals gives fairly consistent evidence that the requirement is decreased when only one vitamin is limiting the growth rate of the animal. In most of the work the diet has been only partially deficient in the vitamin being studied.^{289,235} At certain levels of folic acid feeding, bacitracin, chloromycetin or streptomycin are more active than penicillin or chlortetracycline.²⁸⁸ When none of the vitamin being studied is present, the antibiotics act as a two-edged sword in chicks; the deficiency was aggravated.^{290,291} This is apparently not the case in rats where the antibiotic is helpful even with completely deficient diets.

Occasionally observations have been made wherein an antibiotic completely alleviates the requirement for a vitamin, at least for a short time.^{292,293} Waismann^{294,295} found chlortetracycline would prevent or overcome an antimetabolite-induced folic acid or a vitamin B₁₂ deficiency. This work has not been confirmed, but Nickell²⁹⁶ has shown that any of several antibiotics could substitute for thiamin for several transfers of *Rumex acetosa* virus tumor tissue which normally requires thiamin for growth.

Results with penicillin, chlortetracycline, oxytetracycline and streptomycin are summarized in Table 3-6. In general a sparing action is noted for both B-vitamins and fat-soluble vitamins. Only sporadic work has been done with other antibiotics. Less work has been done with large animals. The low antibody production induced by a pantothenic acid

TABLE 3-6
EFFECT OF ANTIBIOTICS UPON NUTRITIVE REQUIREMENTS*

	Penicillin		Chlortetracycline		Oxytetra- cycline	Streptomycin	
	Rat	Chick	Rat	Chick	Rat	Rat	Chick
thiamin	+	+	+	+	±	+	
riboflavin	+	0+	+	±	+	+	
niacin			+	+	+		
pyridoxine	+	0	±				
biotin	0?	+		+			
choline		+					
pantothenate	+	+0	+		+	+	
folate	0	+	+	+		-	+
vitamin B ₁₂		±	±	+			
vitamin A		+	+				
vitamin D		+					
vitamin K		0					

* Note: + shows sparing action is involved. - shows increased requirement. 0 indicates no effect is observed. ? is given for conflicting or unconfirmed data.

References: 155, 261, 264, 286, 291, 300, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315.

deficiency is stimulated by feeding antibiotics.²⁹⁷ The pantothenic acid requirement of pigs is reduced by chlortetracycline.²⁹⁸ Sources of unidentified growth factors gave the same growth response as antibiotics.²⁹⁹ It would seem to be important to repeat the above animal work to determine the effect of antibiotics upon bacterial vitamin requirements.

Most workers interpret the results with B vitamins in terms of increased synthesis by intestinal bacteria (or less destruction by same). However, they quickly abandon the restrictions of the intestinal synthesis theory when trying to explain the same type of data obtained with energy, minerals, the fat soluble vitamins or protein. To be acceptable now, the explanations must include the cell permeability, the more efficient absorption of the mucosal wall, or better utilization of the nutrient. This is especially true for the calcium requirement which is reduced by penicillin,^{259,316} the manganese requirement which is affected by antibiotics and arsonic acid in chicks,²⁶⁴ and the energy requirements,²⁴³ which are affected by streptomycin. Surely the same mechanism must be working for the B-vitamins that works for protein minerals and fat-soluble vitamins. Such experiments should be repeated in germfree animals in order to clearly differentiate the direct from the indirect action.

11. PRODUCTION OF MILK AND EGGS

No real advantage was seen in feeding antibiotics to dairy cows on a routine basis.^{132,317} Work to date has shown no ill effects in terms of milk production, fat content of milk, bacterial count of milk or feed efficiency of the cow.³¹⁸ Nor was significant change seen in food or water consumption, butterfat produced, pulse rate, body temperature, rumination or general health.^{317,318}

Egg production and fertility were reported to be increased by oxytetracycline, chlortetracycline, or bacitracin,^{319,320,321} streptomycin,^{322,323} penicillin,^{321,323} and arsonic acid.^{324,325} Others report that no difference is obtained with penicillin,^{232,326} oxytetracycline or chlortetracycline^{327,328,329} or streptomycin.³³⁰ Increased hatchability is occasionally seen.^{57,75,323} Decreased egg production and egg weight is also reported.³³¹ Reasons for the lack of unanimity include the idea that positive results are obtained under poor conditions where egg production is low,⁵⁷ temperatures are too high,³³² etc.

The growth of progeny from antibiotic-fed hens is sometimes greater and sometimes less than that of controls^{320,333}; conflicting data were also reported for turkey hens.^{334,335}

Feed requirement per dozen eggs is decreased.³³⁶

12. REPRODUCTION

Although there is very little data to be considered, Ellis⁷⁶ suggests that antibiotics tend to encourage early sexual maturity. Others report no effect of chlortetracycline on data of sexual maturity or fertility.³²⁸ Also pertinent here are the reports on poultry performance given above. Penicillin reduced egg production and hatchability in turkeys.³³¹ Feeding 40 ppm chlortetracycline did not affect reproduction nor was any transfer of antibiotics noted through the placental barrier.⁷²

Reproduction in mice was not affected by 100 ppm of chlortetracycline.¹⁵⁶ The addition of penicillin to the diet of rats has no effect on reproduction when the diet was well balanced.³³⁷ Rats fed 400 ppm oxytetracycline or streptomycin showed no significant differences in reproduction or lactation for two reproduction cycles.^{338,339}

Jukes⁸⁰ and Cunha¹⁰³ review early work on antibiotics in swine reproduction which gives no clear indication of consistent values for the average number of pigs per litter, the average birth weight of the young or the number of pigs born dead. No harmful effects were seen with the low level feeding.³⁴⁰ There is, of course, benefit in viability of young pigs.²⁴⁵ The estrus is not disturbed in swine⁷⁶ nor is weight or survival of embryos affected.

13. LONGEVITY

One report indicates that feeding rats 200–400 ppm of streptomycin or oxytetracycline resulted in a somewhat decreased (10%) longevity.

14. DISEASE

While the effect of antibiotics upon disease and the therapeutic uses of antibiotics are outside the scope of this book, certain relevant general observations are pertinent. Animals which are susceptible to, and exposed to infectious diseases, or which already diseased should be benefited by any drug which prevents or diminishes the severity of the disease. One of the results of successful treatment of infection in growing animals is a rate of growth more nearly approaching the genetic maximum. Therapeutic agents have been added to the water (and/or food) of sick poultry for many years.

Morehouse and Mayfield¹² added arsonic acid to the water of chicks which were exposed to coccidiosis as much to prevent infection as to cure. The increased growth observed in treated birds over that of untreated birds was the expected response; the sick birds did not grow as fast as those in which the disease had been partially or wholly prevented. Antibiotics in poultry feeds are now accepted and are being used for prophylactic and therapeutic purposes.³⁴¹

Jukes⁸⁰ has reviewed the work of Catron, Carpenter, Cunha and others in establishing the concept that the growth response to dietary antibiotics varied with the disease level in pigs. He concludes that antibiotics relieve the conditions of subacute infections and diarrhea, and that even in apparently healthy animals, the effect is mediated by the elimination of harmful bacteria. Whitehair³⁴² noted that Cesarean born baby pigs reared in isolation did not respond to antibiotics. This conclusion is supported by the evidence of Coates *et al.*,³⁴³ who find that chicks grown in old (dirty or bacteriologically contaminated) quarters have a retarded growth rate when compared to chicks fed antibiotics or chicks maintained in clean quarters. The growth of chicks reared in new quarters was not improved by the levels of antibiotics tried.

Growth of chicks in clean quarters was reduced when gut contents from chicks in dirty quarters were fed to them. Heat sterilization of this same material rendered it ineffective. Thorough cleaning of the premises irradiated the growth retardation effect for a short time, but it re-established itself. Cooper and Gordon³⁴⁴ found that they could transmit this "agent" either orally or intranasally by using crude filtrate of lung tissue.

The incidence of cervical abscesses was reduced in pigs fed 50–100 ppm of antibiotic.³⁴⁵ Urinary calculi were reduced in lambs fed chlortetracycline.²⁵⁸

The ability of dietary antibiotics to increase growth rates of both chicks,^{33,84} and pigs³⁴⁶ had decreased over the years as the growth of unsupplemented groups improved from 1950 to 1955. This effect is attributed to a suppression of harmful bacteria. Although it appears to be obvious that dietary antibiotics increase the growth rate of animals with infectious diseases of both clinical and sub-clinical nature, many experiments with positive results are cited under Modes of Action of Antibiotics in this Chapter, wherein no deleterious effect due to bacteria was apparent.

Antibiotics may be helpful not only in frank disease, and general malaise, but also in abnormal intestinal microflora problems, "sub-clinical infection," and even in the normal every-day symbiotic, commensurate and parasitic shift between the host and its intestinal microorganism. This general concept has been well expressed by Dubos.³⁴⁷ "The evaluating equilibrium corresponds to a compromise between many conflicting necessities. On the one hand, for example, the presence in the tissues of large numbers of relatively innocuous microorganisms helps in controlling the multiplication of other species with great pathogenic potentialities—from fungi to cholera vibrios. As we have seen, elimination of the autochthonous flora by drug treatment leaves the field opened to many kinds of infection of either endogenous or exogenous origin. On the other hand, many of the microorganisms normally present in the tissue have mild deleterious effects which, although compatible with a normal life, do interfere somewhat with the optimum performance of metabolic functions. Any therapeutic procedure which eliminates, or merely attenuates, these deleterious influences thereby brings indirectly greater metabolic efficiency."

Radisson *et al*³⁴⁸ found no change in the phagocytic activity of leucocytes of calves fed chlortetracycline, while bacteria isolated from feces of those calves were more susceptible to phagocytosis than bacteria from control calves. This would suggest that antibiotics are most valuable in young animals between the time when the antibodies contributed by the dam are decreasing, and before the animal's own antibody-making machinery is in full production.

15. HORMONES

Little positive data are available for review of the effect of dietary antibiotics upon hormones.

Diethyl stilbesterol is apparently used in the many calf feeds and concentrates for steers. The effect of this and antibiotics may or may not^{349,350} be additive. Additive effects have been reported for thyroxine and antibiotics in increasing growth rate.³⁵¹ The addition of both thyroxine and stilbesterol gave improvement in feed efficiency with a diet

containing penicillin.³⁵² This appears to be a fruitful area of research today.

16. TOXICITIES

Detrimental effects are sometimes seen after feeding antibiotics to animals. Some of these are seen consistently and others rarely. Most minor changes evoked in the host are discernable only to the specialist. The extensive continued use of antibiotics in this country is ample proof that pharmaceutical firms and the feed manufacturers have cooperated to give the farmer a product with economic advantage which avoids serious toxic effect.

Large quantities of antibiotics may be harmful to ruminants³⁵³ which are not sick.

Although Jukes⁸⁰ notes that pigs have been fed 1000 ppm of a combination of antibiotics with no harmful effect, feeding antibiotics presents new problems in pigs for the veterinarian,³⁵⁴ such as super infections. One report indicates that dermatitis and weight loss may be seen with only 5 ppm of streptomycin.³⁵⁵

Chicks have been fed 10,000 ppm oxytetracycline for one month with no obvious deleterious effect.³⁵⁶ Lower levels of antibiotics have been reported to cause leg weakness^{357,358} or prolonged blood-clotting time. Evidently the former is rarely seen and the latter is not serious.³⁵⁹

The increased incidence of yeast seen in the intestine of animals fed antibiotics¹⁸⁸ may be caused to some extent by the growth stimulation of candida by the drugs. Animals inoculated with pathogens show increased fatality when antibiotics are fed. This effect is discussed with *in vivo* hormesis later in this chapter.

Some reports¹⁸⁹ suggest that antibiotics are especially toxic to guinea pigs—presumably due to the increased activity of *Listeria* in the presence of the drug. However O'Dell *et al*³⁶⁰ reported a beneficial effect from feeding chlortetracycline to a stock colony of guinea pigs.

17. DISCUSSION OF EFFECTS

A survey of all of these striking effects found under well controlled conditions where the only known variable was the presence or absence of antibiotics, brings to light the great variety and extent of changes produced by such small amounts of biologically active compounds. From the biological truism that everything affects everything, the question that immediately arises is which of these effects were first and which caused others. For every change evoked, a balance mechanism is brought into play; a simple stimulus evokes a response with many harmonics and over-

tones. The next question is whether other biologically active compounds could cause a similar complexity of response, or are antibiotics unique in this respect? One suspects not; a single vitamin or even a small quantity of radiation can affect as many systems in as many ways. How many detailed changes one could find after cortisone injection!

How can all these changes be caused by a single entity—what is the exact mechanism through which any change is mediated in the growth or metabolic pattern of cell, tissue or organism? A satisfactory answer to this question must lead to a series of chemical equations with the antibiotic as one of the molecules contributing to the reaction rate.

E. MODES OF ANTIBIOTIC STIMULATION

One of the most intriguing questions raised by antibiotic feeding is the exact mechanism by which they act in low concentrations to increase growth rates. The subject should be approached not with the expectation of finding a single mode of action to account for the results, but to determine to what extent each of several modes is responsible for the results obtained under a given set of conditions. The methods of Snell *et al*³⁶¹ on the modes of action of antibiotics in killing bacteria may be applicable to the modes of action of antibiotics in growth stimulation.

The formidable array of suggestions which have been proposed as modes of action of antibiotics are given below in outline form. Since it would be tedious to consider each one separately, their documentation and discussion follows a more general pattern. Evidence for many of the proposals has already been given. Some are presented as logical proposals with no direct references to the literature. Moreover, these proposals are not mutually exclusive. Many suggestions listed under direct action could be components of indirect mechanisms, and *vice versa*. The list will fulfill its function if the concepts implied are found to be useful in the elucidation of this problem, or to stimulate further work in molecular biology.

1. PROPOSED MODES OF ACTION OF ANTIBIOTIC GROWTH STIMULATION

a. Indirect Action:

a-1. Via intestinal microflora:

I. Increase numbers of "good" microorganisms:

- a) Vitamin synthesizers
- b) Over populate (potential) pathogenic organisms

II. Decrease numbers of "bad" microorganisms:

- a) Vitamin users
- b) Toxin producers
- c) Pathogens or potential pathogens

III. Change organisms present:

- a) Produce (resistant) strains which are less harmful
- b) Change metabolism of those present
- c) Alter energy requirements in the rumen
- d) Decrease invasiveness of normal flora
- e) Increase susceptibility to phagocytosis

IV. Relocate organisms into their usual habitat (prevent rising of lower gut organisms).

a-2. Reduce infectious diseases:

- I. "Sub-clinical" infection
- II. Help body defenses generally
- III. Decrease frank infectious disease

a-3. External (or intestinal) milieu reaction:

- I. Detoxication (chelation)
- II. Remove inhibitor (metabolic waste product)
- III. Chelation-activator
- IV. Reduce surface tension
- V. Reduce pH of intestinal milieu

b. Direct Action:

b-1. Cells:

- I. Permeability of cell wall
- II. Biological stabilizer against stress
- III. Protoplasmic stimulant
- IV. Activate anabolic regulator
- V. Mutagenic agent (microorganisms)
- VI. Mitotic stimulant
- VII. Stimulate production of cell wall material
- VIII. Adaptation expeditor

b-2. Tissues:

- I. Intestinal wall length, weight and thickness made more efficient
- II. Increased absorption rate
- III. Increased apparent utilization of metabolites
- IV. Decreased energy expenditure

b-3. Organism:

- I. Hormone synergism
- II. Increased growth or thyroid hormone
- III. Increase palatability
- IV. Over reaction to stimulant
- V. Increase food utilization
- VI. Increase adaptability to a poor environment
- VII. Decrease sensibility to poor environment
- VIII. Biological stabilizer to stress
- IX. Adrenal cortex reaction

b-4. Metabolic reactions:

- I. Decrease vitamin requirement
- II. Increase vitamin synthesis by tissues
- III. Act as a metabolite
- IV. Stimulate specific reactions:
 - a) Photosynthesis
 - b) Sucrose synthesis
 - c) Vitamin A from carotene
- V. Produce less (toxic) side products
- VI. Increase enzyme synthesis

c. Combination of several of the above

d. Non-specific activation giving general mobilization of metabolic enzymatic potential abilities.

2. INDIRECT ACTION VERSUS INTESTINAL MICROORGANISMS

In 1885 Pasteur³⁶² suggested that animal life as we know it would be impossible were it not for all of the good microorganisms in the intestinal tract. One year later Nencki³⁶³ contested this statement with the comment that intestinal bacteria produce toxins such as skatole. While Metchnikoff³⁶⁴ championed the cause of the lowly lactobacilli during the following decade, Osborn and Mendel³⁶⁵ contributed the first direct evidence on the subject. They found that, on a diet low in amino acids and vitamins, rats which were allowed coprophagy grew much better than rats which were restricted from their feces. This reaction was attributed to material synthesized by the intestinal microorganisms. Although animals have been reared in a completely germfree environment (reviewed by Luckey¹⁶) the idea of Pasteur and Metchnikoff persists as the *Intestinal Synthesis Theory*.

a. Intestinal Synthesis Theory

The intestinal synthesis theory states in general terms that intestinal microorganisms produce compounds from materials available in the intestinal tract, which, by virtue of cellular secretion, excretion, or breakdown, become available to the absorptive phenomena of the host; the compound is then absorbed and utilized by the host. Despite the fact that direct proof of the whole thesis is lacking for monogastric animals (only in the *gastric* part of ruminants is there acceptable data), nutritionists use this catch-all theory for any effect which is not immediately explicable. Bacteria exist in the intestine, and there is good evidence regarding the ability of some of these organisms to produce vitamins, amino acids, etc. from simpler compounds. However, there is some question regarding how much material synthesized may be released from the bacterial cell into the intestinal lumen. There exists no direct evidence that the compounds produced are utilized by the host. The specific problem is then how much, if any, material produced by intestinal microorganisms is utilized by the host. Most people assume as much to be absorbed as is needed to explain their results.

This theory was immediately invoked to explain the phenomenon of increased growth in a situation where classical considerations allowed the prediction that decreased growth should have been seen.¹¹ Several possibilities were suggested, to the effect that the antibiotic altered the microbial population or the metabolism of the organism present to (a) produce more of a nutrient (vitamin) which was limiting growth of the animal, or to (b) produce less of a "toxic" product which had been inhibiting the growth of the animal or to (c) use or immobilize less of an essential nutrient.

b. Changes in the Intestinal Flora

As pointed out earlier in this chapter, little or no uniformity exists in the observations wherein intestinal flora changes were examined in animals fed antibiotics. Gordon and Taylor³⁶⁶ suggested that the essential action of antibiotics takes place in the upper portion of the gastrointestinal tract, since penicillin is largely destroyed or absorbed before it reaches the lower intestine. Freerksen³⁶⁷ suggested that antibiotics keep lower gut organisms from rising in an oral direction. Such explanations still leave unanswered the question as to why so few antibiotics are really effective in promoting growth.

Antibiotics may encourage the growth of detrimental organisms; the guinea pig is most sensitive to antibiotics and dies while the cecal flora change to more harmful organisms. Eyssen *et al*³⁶⁸ suggest that this

effect is a destruction of normal intestinal flora which results in a deficiency of an essential growth factor and anabolic failure. The fact that guinea pig can survive without such help is shown by the survival of germfree guinea pigs.³⁶⁹

Antibiotics may encourage beneficial flora, as is indicated by the positive growth response obtained by feeding cultures of different living organisms in an attempt to alter the flora or to supply a nutrient.³⁷⁰

c. Feeding Bacteria

Several workers report increased growth in poults and chicks fed *E. coli*, some of which were isolated from the ceca of chicks fed penicillin.^{371, 372, 373, 374} Others reported no growth response from feeding either *E. coli*,^{375, 376, 377} or an *aerogenes* culture.

Kratzer *et al*³⁷⁸ found a ten-fold increase in yeast in the intestine of turkey poults fed antibiotics. When this last culture was fed to chicks or to poults, no equivalent increase in growth was noted. Certain selected strains of yeast, fed at 0.1% level, did give an increased growth rate in poults.

Symster *et al*³⁷⁹ found that the number of *Clostridium welchii* increased in the intestine of chicks fed penicillin. Williams *et al*³⁸⁰ stated that feeding this organism or toxins produced by it, gave no growth depression, nor did antitoxins from a variety of *Clostridia* give a growth effect.³⁸¹ Elam *et al*³⁸² fed spores of *Cl. welchii* to chicks which produced a growth depression. Lev *et al*³⁸³ found chicks in clean quarters had no *Cl. welchii* in their intestinal tract while those reared in dirty quarters had the organism in their tract. This organism was eliminated from the gut when penicillin was fed. Penicillin also overcame the growth depression induced by feeding fecal clostridia to chicks.³⁸⁴ Feeding *Asperigillus* cultures stimulated the growth of swine and chicks.³⁸⁵ Such indirect evidence is used to suggest one mode of action of antibiotics is to reduce the amount of bacterial toxins being absorbed by the chick. However feeding antibiotics with living gram-negative organisms may give better growth than either alone.³⁸⁶

Since neither microbial population studies nor feeding suspected microorganisms gave clear evidence in favor of the indirect action hypothesis, several workers suggest the avenue that a changed metabolism in some of the intestinal micro-flora should be examined.

1. Changed Metabolism of Intestinal Microorganisms

François³⁸¹ and his collaborators find a direct correlation between the growth stimulation provided by different antibiotics and their ability to

inhibit *in vitro* amino acid deamination^{388,389}—thus prevent “a chronic toxicosis due to ammonia.” Unfortunately they have not been able to relate the amount of ammonia produced *in vivo* quantitatively with ammonia toxicity studies. François also reports a relationship between the growth stimulating antibiotics and their ability to decrease the production of trimethylamine from choline by bacteria.^{387,390} Dintzis and Hastings³⁹¹ have shown that chlortetracycline would depress the microbial decomposition of urea in the stomach of mice. Similarly chlortetracycline is found to suppress the formation of amines by the intestinal flora, as well as to produce a decreased amino acid decarboxylase activity of mixed *in vitro* fecal cultures.²⁴⁴ Increased resistance of certain groups of microorganisms to the drug being fed is expected, and noted.³⁹²

e. “Sub-Clinical” Infection

Chicks reared in new clean quarters were found to grow better than chicks in old quarters, and whereas chicks in old quarters responded well to antibiotics, those in new quarters responded poorly, if at all.^{393,394} Coates and her colleagues extended this work by placing chicks in sterile plastic cages.³⁹⁵ These chicks did not respond to antibiotics unless they were inoculated with fecal material from chicks reared in “dirty” quarters. The inoculation generally reduced the growth rate, and feeding antibiotics overcame this inhibitory effect. However, Hill *et al*³⁹⁶ found that increasing the level of antibiotics to 220 ppm did provide an increased growth rate in clean chick quarters. Speer *et al*¹⁰⁸ had performed similar experiments in pigs with similar results in 1950, and Whitehair and Thompson³⁹⁶ found no response to antibiotics in baby pigs reared in a “disease free” environment. In contrast to these results Hill and Larson³⁹⁷ found that pigs obtained by hysterectomy and raised with no contact with other pigs or pig infections, grew at a faster rate when fed chlortetracycline than the group fed no antibiotics. Calves reared in clean quarters respond to dietary or injected antibiotics more quickly than those reared in old quarters.³⁹⁸ In this experiment no difference in “infection level” was indicated by white blood cell counts.

Nevertheless the decreased diarrhea and scouring seen in pigs fed antibiotics¹⁰⁵ suggests that their presence inhibits the activities of harmful microorganisms in the intestinal tract. If a drug stimulates growth indirectly by its action on the intestinal flora, Briggs³⁹⁹ suggests that it be termed a promotant.

Freerksen³⁶⁷ gives specific and plausible substance to the will-o'-the-wisp “sub-clinical infection”—with evidence. He suggests that oral antibiotics help physiological mechanisms (such as peristalsis, pH, cel

structures and the cleansing action of a food going through the intestine) to keep the rectal flora from advancing into the upper sections of the intestines which are flooded with microorganisms from the lower intestine during periods of poor health, poor sanitation, or stress (as even in confinement). His attractive hypothesis states: "By supporting the self-purification process antibiotic supplementation leads to a restoration of the normal functions of the stomach and the upper intestine, normalizing, perhaps as a consequence, certain metabolic processes. These metabolic readjustments, therefore, are not a direct effect of the antibiotic or the crude fermentation produce. This action of antibiotics is not, of course, restricted to growing animals, but in growing animals the effect is more noticeable; first, because improvement in the general condition is shown by the easily measurable gain in weight, and second, because very young animals are more susceptible to unfavorable environmental conditions."

One mode of action of dietary antibiotics may well be an increased ease of absorption of nutrients with the decreased proportional effort of the intestinal wall to defend itself when antibiotics are present. Although there appears to be no real decrease in the total microbial population, nor any consistent change in the bacterial groups when antibiotics are fed, the evidence strongly suggests the presence of antibiotic effects a decisive change in the balance between the host defense mechanism and the constant offensive action of the microbes to intrude through the barriers of the mucosal membrane. That organisms may become less virulent is seen in tuberculosis bacillus treated with antibiotics. Organisms may be more susceptible to phagocytosis.³⁴⁸ The metabolic director of the activities of microorganisms seems to have ordered a retreat from the usual guerilla warfare with the goblet cells in and about the villi of the intestine, to a position of metabolic defenses against an apparent micro-newcomer on the scene, which evidently produces a weapon to be reckoned with—the antibiotic.

Evidence from the host indicates that the myriad micro-raids have effectively ceased. The presence of antibiotics in the intestine in some way prohibits the microorganism from penetrating the defense mechanism of the wall to activate the line of secondary defense. The need for phagocytosis is decreased materially; the size and lymphocyte count of the illeocecal tonsil is decreased to about one-half of the level found in chicks fed no antibiotic.¹⁷¹ The second evidence provided by the host is the decreased weight or thickness of the cell wall. The main difference, on histological examination, appears to be in the decreased quantity of connective tissue present in the intestinal wall of chicks which have received antibiotics. This intestinal wall contains primarily the cellular elements needed for absorption; little of the elements necessary for de-

fense are seen.¹⁶⁶ The excess connective tissue in the wall of conventional chicks (which is not seen in germfree birds which have no stimulation from bacteria) may be scar tissue from repeated encounters with bacterial invasions. This wall must expend relatively much energy, metabolic effort and morphological preparation in defense and repair; antibiotics allow economy in these fields and thus relatively more of the gut wall of antibiotic-fed chicks is functioning primarily in absorption. Since many absorptive processes involve energy-requiring reactions, absorption is most efficient when the cells specialized for this purpose are present in full complement. Dilution of them by excess collagen, micro scar tissue, or lymphocytes can only decrease this function.

f. Infections

The correlation between bacteriostatic detectable levels of antibiotics in body fluids and the growth response obtained has suggested that dietary antibiotics may act in the prophylactic sense in distinct contrast to the therapeutic use. The chief action of dietary antibiotics may be to be present continuously in bacteriostatic concentration to aid the normal defense mechanism of the body in preventing the establishment of either sub-clinical or clinical infections. This would include the "showers" of bacteria found in blood after chewing. Dietary chlortetracycline-protected calves were infected with *E. coli*. Of 25 calves in each group, 14 died with *E. coli* bacteremia in the control group and only 4 died in the groups of 25 fed antibiotic. On a practical basis fewer disease problems are encountered by the veterinarian.⁴⁰⁰ Antibiotics are not always useful for bloating in cattle.^{400a}

Of similar nature appears to be the case encountered in the experiment of Morehouse and Mayfield in which arsenic acid in the drinking water protected chicks infected with coccidiosis,¹² and also that in the experiment of Riedel *et al*¹³ wherein quaternary nitrogen compounds helped the growth of chicks infested with round worms. The medical and veterinary records of the past decade give more than adequate proof that antibiotics are helpful in many infectious diseases. High level feeding is, apparently, economically successful prophylaxis which allows better health survival and growth in treated animals. In some way our use of antibiotics does lead to development of a few resistant strains of bacteria.^{400b}

3. DIRECT ACTION OF ANTIBIOTICS

a. Animals reared in clean quarters

Moore *et al*¹¹ suggest the possibility that antibiotics act directly or systemically. Aside from the negative evidence that workers who have

looked for the "infectious agent" have not isolated it, and cannot agree on the changes evoked by antibiotics, the following indirect evidence of the possibility of direct action has accumulated. Changes in the intestinal flora are seen when no growth stimulation or change in the flora is found.¹⁷¹ Bartley *et al*⁴⁰¹ found calves reared in new quarters responded to antibiotic feeding, as had those in quarters where colds and scours were prevalent. Hill and Larson³⁹⁷ found that Caesarian-born pigs reared in clean isolation units grew at a faster rate when chlortetracycline was fed. These pigs were free from all contact with other swine and showed no recognizable swine disease.

Penicillin prolonged the survival time in mice given lethal injections of trypan blue.⁴⁰² Rimocidin (ineffective against bacteria) caused increased growth rate in pigs.⁴⁰³ The highly insoluble Carbomycin-B stimulated growth in chicks, but had no effect on the intestinal microorganisms when administered intraperitoneally.⁴⁰⁴

3. Injected Antibiotics

Many experiments wherein antibiotics were injected have given either negative or less positive results than are usually obtained by oral administration. However, the scattered minority cannot be excluded from consideration, since a thousand negative experiments ring less true than one or two positive results. We shall therefore accentuate the positive.

When the equivalent of 15 ppm of antibiotic was given daily by intramuscular injection (0.5 mg/kg body weight), the concentration of antibiotic found in the small intestine was equivalent to that produced by feeding a diet containing 2-4 ppm. Gordon⁵⁸ concludes that "the growth response to penicillin was exactly the same by injection as by mouth. In other words, the level of antibiotic in the digestive tract following injection was not sufficient to bring about the growth response which was recorded, thus suggesting that the site of action need not necessarily be confined to the digestive tract."

Calves injected with chlortetracycline grew faster⁴⁰⁵ than controls, although no detectable antibiotic could be found in the rumen.⁷⁰

Bruggemann⁴⁰⁶ reports daily parenteral administration of antibiotics to rats promoted growth similar to that produced by oral administration in the species. Waisman *et al*²⁹⁴ find that injected aureomycin counteracts aminopterin-induced folic acid deficiency in rats.

4. Inactivated Antibiotics

Several workers have reported growth stimulation with inactivated antibiotics. These data are of real importance in the support of the idea

that antibiotics can act directly in the absence of bactericidal action by the compound. Bacteria are not killed by these derivatives, nor are they treated as the parent compound in the induction of resistance or of changed nutritive requirements by the microorganisms.

When Elam *et al*^{407,408} injected autoclaved (inactivated) penicillin into chicks, they grew at a faster rate than control chicks. The autoclaved penicillin gave no response when fed with the diet. Fell *et al*⁴⁹ found that DL-penicillamine gave a growth response when injected but not when added to the diet. Williams *et al*⁴¹⁰ and others⁵⁴ confirmed the work of Elam by showing that steam-inactivated penicillin retained its growth promoting ability, although it had a lessened potency. Jukes⁸⁰ reports consistent growth stimulation with penicillinase-hydrolyzed penicillin, and to a lesser extent with D-penicillamine.

Taylor and Gordon⁴¹¹ provide provocative proof that penicillin stimulates growth even when inactivated. Penicillin inactivated by heat, penicillinase or heavy metals, produced growth stimulation in pigs when administered either by mouth or by injection. This material had no antibiotic activity.

d. Stimulation by Non-antibiotics

Many compounds which are not antibiotics, and which have little bacteristatic or bactericidal action, have been reported to give growth response when fed to animals in small quantities (this report eliminates any necessity for discussion of such compounds as ascorbic acid and pentoses which, when fed in larger amount, stimulate the growth of chicks). Most of this work has been done on arsonilic acid and its derivatives in poultry growth stimulation, as reviewed by Frost.⁴¹² There is no correlation between the bacteriostatic and growth-promoting actions of this series of compounds. They seem to function best, as do antibiotics,⁴¹² when a poor protein such as soybean oil meal is fed.⁴¹⁴

The following compounds stimulate the growth of chicks: sulfasuxadine^{10,11} arsonilic acid⁴⁰ or its derivatives,²⁶⁴ furazolidone,¹⁷⁴ possibly gibberellic acid,^{414a} and surfactants of different types.^{264,415,416,417a,b,c,d} The growth of turkey poults is accelerated by arsanilic acid.⁴¹⁸ Swine growth is stimulated by arsanilic acid,^{41,419} sulfa drugs,⁴²⁰ surfactants^{200,421} and copper (250ppm).^{422,423} Increased feed efficiency is also noted in swine when fed arsonilic acids.^{424,425} Non-antibiotic surfactants²¹² and p-amino salicylic acid⁴²⁶ have also been reported to increase the growth rate of calves.

Analysis of swine fed surfactants indicated the same carcass changes were found in antibiotic-fed swine.²⁰⁰

Arsenic compounds (Fowler's solution, etc.) have been used for many decades in veterinary practice to treat general malaise, unthriftiness, and poorly defined conditions where the animal showed no clinical disease. The familiar pattern of a drug reducing vitamin and nitrogen⁴²⁶ requirements has been seen in chicks fed arsonilic acid. A variety of arsonic acids accelerated growth in chicks fed diets high in raw soybean oil meal.⁴¹⁴ Feed efficiency is increased, gut length is decreased,⁴¹² egg production and hatchability are increased.³²⁵

e. Action via Hormones

Any effort to discover a single mode of action for the growth stimulation of the wide array of species that includes microorganisms, plants, silkworms, chickens, rats, mice, pigs and man must eventually take into consideration the factors which are constant throughout. It does not seem that any single, typical flora or even, in some cases, any flora can be a constant factor. On the other hand, the common factor can well be metabolism, since a similar pattern of chemical composition and molecular metabolism exists in all living forms. Another possibility is that of the hormones, which would exclude the microorganisms unless the term hormone is redefined, or a new term is used to include the growth and metabolic regulators which affect the living cell that produces it. Is not insulin a "hormone" in the β cells of the Isles of Langerhans of the pancreas? Is not thyroxine a "hormone" before it is secreted by the cell which produces it? Do not "hormones" regulate reaction in the mother cell? The answers to these questions may well be in the affirmative. The word parahormone has been suggested as a name for these substances in order to express this property.

A potentiating action on anabolic reactions via the growth hormone, or a retardation of the function of catabolic regulators such as cortical steroids⁴²⁷ or thyroxine,⁴⁰⁶ might easily account for increased growth rate in animal species. Chlortetracycline and penicillin (K salt), fed at a level of 1 mg/kg body weight, increased the size of the thyroid in rats and decreased the I^{131} uptake. The observed goitrogenic action simulated that of thiouracil.⁴²⁸ Penicillin increases the growth rate of rats which were stressed by feeding them 0.2% protomone.⁴²⁹

Sex differences are seen in the coloration of pork muscle,¹⁰⁶ and occasionally in growth rates of birds.^{226,307,430,431,432,433} Female rats do not respond as male rats do to antibiotics fed with tyryptohpane-low diets.²⁸⁷

Antibiotics appear to act synergistically with plant hormones in growth and resistance to disease, possibly by increasing cell permeability.

The work with hormones may best be summarized by noting that it

has not begun to be developed as a fundamental tool in antibiotic research.

Antibiotics act as biological stabilizers in animals—possibly by releasing or stimulating adaptation mechanisms. They help the organism to fit into the balance of nature. The homeostatic mechanism may be as simple as the presence or absence of a molecule, or as complicated as a society. Size, growth rate, reproduction rate and longevity of invertebrates may be predetermined by the number of organisms present, relative to the size of the container.⁴³⁶

f. Antibiotics in Stress

Antibiotics have been found to have a beneficial effect upon animals under a variety of stress situations.

Chlortetracycline was found to increase the growth of rats under conditions of alloxan diabetes, although more sugar was excreted per day by the antibiotic-fed rats.⁴³⁷ Increased survival was noted in antibiotic-fed rats subjected to shock via drum rotation. The action was partially explained by the decreased release of vasoactive ferritin from the liver of the chlortetracycline-treated animals.⁴³⁸

Oral administration of antibiotics is effective in producing resistance to the irreversible shock induced by hemorrhage in dogs,⁴³⁹ and it is also effective in increasing resistance to radiation injury.⁴⁴⁰ Both of these conditions have a bacterial invasion vector which has thus far prevented proper evaluation of any direct effect which antibiotics may have. Zweifach^{440a} suggests a direct action is involved since germfree animals react to hemorrhagic shock in the same manner as do conventional animals.

Gyorgy⁴⁴¹ has found that antibiotics delay the appearance of massive hemorrhagic necrosis of the liver in rats fed a low-protein, low-methionine, vitamin E-free diet. The antibiotics also increased the growth rate 3–5 fold in these animals. Since the liver damage has been found in rats which were germfree,⁴⁴² bacterial activity in this syndrome is doubtful. Chlortetracycline or choline prevented renal lesions in rats fed a low-choline, high-fat diet.⁴⁴³ Castrated rats showed a growth response to oxytetracycline, while normal rats did not,¹⁷⁰ indicating again that antibiotics act most efficiently under conditions of stress. Chlortetracycline counteracted the inhibition of growth, alopecia and thymus atrophy seen in rats injected with cortisone.⁴⁴⁴

Antibiotics appear to be more effective for chicks in cold weather or hot weather when they are on low energy diets⁴⁴⁶ than when they are fed high-energy diets. Antibiotics promote growth under conditions of dietary stress, such as when they are fed a vitamin-low diet²⁹² or low protein

diet,⁴⁴⁶ more than when the diet is balanced. Under the stress of a high-fat diet, female turkey poults responded to penicillin (15 mg/kg), while male poults showed no effect.⁴³³

Penicillin reduced markedly feather picking in chicks fed a high fat diet,⁴⁴⁷ and counteracted the growth retardation seen in chicks fed 10% raw soybean oil meal.⁴⁴⁸

There appears to be a direct relationship between the amount of stress existing for the animals and their reaction to antibiotic feeding. The type of stress does not seem to be important; it may be a partial food deficiency; a change in environment, heat or cold, respiratory or digestive disease, overcrowding, or the newborn state. All these conditions seem to bring an animal to a state in which a greater positive reaction to antibiotics is seen. The drugs seem to help adaptation in those individuals which have apparently adapted least. It has been suggested that the antibiotics may prepare the animal for stress.⁴⁴⁹ The slight growth stimulus provided by antibiotics in animals under good dietary and environmental conditions may indicate that the genetic potential for growth has been approached. Such a view may dampen many research efforts, but its implications must be considered sooner or later.

g. Germfree Experiments

The proper approach in seeking a direct action of antibiotics is to feed the drugs to animals which have no microflora. When antibiotics were fed to germfree chicks and turkey poults,^{356,450} the results indicate that low levels of antibiotics do stimulate growth, while high levels have a slight depressing effect. When 11 mg of procaine penicillin were fed (4 chicks per group), the growth index was 103 ($P = 0.003$). When 25 mg oxytetracycline was fed (13 ± 1 chicks per group), the chicks grew at a significantly faster rate than germfree chicks fed no antibiotics ($P = 0.001$). Moreover, 14 germfree turkey poults grew at a faster rate ($P = 0.003$) when fed procaine penicillin than did 13 control germfree poults fed no antibiotics. One may presume from these data that antibiotics do act directly upon the animal *per se*. The fact that higher concentrations were not effective is shown in the next section of this chapter to fit the expected pattern of action of a hormoligant.

The optimum amount of antibiotic to be fed to germfree birds is probably 1–15 ppm of penicillin and 3–20 ppm of oxytetracycline. The work of Luckey *et al*³⁵⁶ extends into this range, but unfortunately Forbes *et al*⁴⁵¹ found no increase with 25 ppm of penicillin and the work of Gordon *et al*¹⁷¹ was done with larger quantities of antibiotics. Their results were

negative, as would be expected from a consideration of the theory of hormoligosis (as developed in the next section).

Jukes⁸⁰ reports an experiment wherein embryonated eggs were apparently injected with 0.4 or 2.0 mg of antibiotic on the 7th, 8th and 9th days of incubation. Since the egg contents weighed about 50 gm, the low concentration used was equivalent to 8 γ per gm of egg contents. While this level is bacteristatic, it is probably too high to show any stimulation according to the hormologic hypothesis. No positive growth response was seen. Another possible explanation is that the embryo is growing at full genetic capability in an optimum environment, and therefore cannot be stimulated.

The data from germfree chick experiments consistently falls into the pattern, with only a small area where very small quantities of drug give a stimulation in growth. Such a phenomenon is in accordance with the expected stimulating action of drugs given in minute doses. In pharmacology this action is known as the Arndt-Schultz rule; various bacteriologists such as Richet⁴⁵² worked with this effect and recognized it as a general law. Southam and Erlich termed the action *hormesis*.⁴⁷⁵ Botanists find the same action in plant physiology, due to antibiotics and other drugs.²⁹⁶ A proposed name for such action is hormoligosis.³⁵⁶ These doctrines are elaborated here to illustrate the central theme as applied to antibiotics.

Although a discussion of homeopathy, as such, is not pertinent here, one aspect of it which is related to one of the three doctrines of Hahnemann,⁴⁵³ is of interest. His suggestion in 1810 that drugs increase in potency with dilution does not appear to be true generally, his results appeared to be subjectively obtained, and his dilutions were astronomical in magnitude ($1:1 \times 10^{21}$), but the general idea that drugs may have a dynamic effect when used in small doses is similar to the other effects considered here.

The evidence for the direct action of antibiotics strongly suggests hormesis as one of the main vectors in growth stimulation of animals by low level feeding of antibiotics. Animals reared in clean quarters sometimes grow at a faster rate than when antibiotics are added to the diet (higher levels may be needed). Antibiotics stimulate growth when injected intramuscularly or intrapartioneally, while little or none can be found in the intestinal tract. Penicillin can be bacteriologically inactivated and still retain activity when fed or injected. Antibiotics which have no typical antibacterial activity stimulate growth rate. Many compounds other than antibiotics, and sometimes antibiotic derivatives, stimulate growth; still others will undoubtedly be found when they are presented in the proper concentration. The action of antibiotics on

nutritive requirements for nitrogen, minerals and vitamins (particularly the fat soluble vitamins) leaves much room for explanation on a basis other than via intestinal synthesis. The interaction seen between antibiotics and hormones, the increased absorption of certain nutrients, the increased enzyme activity found in the intestinal wall, and increased antibody potential under certain conditions, all suggest a direct action on the tissue of the host. The general observation that antibiotics are most effective under conditions of stress indicates a direct action. Data from feeding germfree animals low levels of antibiotics is clear evidence of a direct action.

A direct action of antibiotics in animals seems to fit the pattern seen in pharmacology and bacteriology. Certainly cells in different species, whether bacterial, plant or animal, exhibit some of the same basic biological reactions. To understand properly this aspect of the growth-stimulating action of antibiotics, hormology should be examined from a more general viewpoint.

F. HORMOLIGOSIS

Hormoligosis designates the stimulatory effect of a very small amount of an agent upon living organisms. The term is derived from the Greek: *hormaein*, meaning to excite; and *oligos*, meaning small. Hormology is the general study of excitation and stimulants. Hormesis was defined as being the stimulatory action of subinhibitory amounts of a toxin, and hormetic is the adjective which describes this action. These useful terms designate the best substantiated vector of hormoligosis. There are, however, hormoligants which are not toxic compounds. Evidence for some of these is presented later in this chapter to illustrate the breadth of the phenomenon.

If any of the data examined above are accepted as indicating that one of the modes of action of the antibiotic stimulation of growth rates of animals is a direct effect on tissues, then one may inquire to see whether this action is a general reaction which affects other living cells. The following evidence indicates that the growth promoting action of antibiotics is a part of a general pattern—a basic biologic reaction of living cells under certain conditions. As such the reaction in animals could have been predicted and may be greatly extended. The action has been elucidated for poisons and metallic ions. Possibly it may apply to many non-metabolites which are not poisons. A stimulatory action by a very low level of a metabolite is difficult to differentiate from the normal metabolic function of the compound. Hormones may belong to a special class of hormoligants produced by cells. These compounds (the hormones) have a relatively specific stimulatory action. This specificity of

action may be considerably less in the plant hormones than in the animal hormones. Nerve cell releases (acetylcholine) or parahormones such as lactic acid may be active. It is quite possible that heat, light, X-radiation, electricity and other forms of energy may elicit the stimulatory response if given in minute doses at the proper time intervals. A small amount of cold or injury or even of psychological factors may eventually be seen to stimulate organisms. A similar phenomenon may be involved in the effect of the size of container on growth, reproduction, or longevity of invertebrates.⁴³⁶

Hormology asks that the complete action spectrum of a compound be examined with particular care to observe the increased potential of living cells in the presence of minute quantities of the stimulant. The cell may act in many ways faster or better in the presence of a minimum stimulus. It has a greater ability to adapt to the specific environment of the moment when it is so stimulated. The activity spectrum of the compound will vary with the conditions, as do all other biological entities; and for this reason exact amounts for a given stimulation cannot be given unless all the conditions are fully specified.

All stimuli may not be hormoligants. However, most highly toxic compounds or harmful physical entities appear to be. Any stimulus, smaller than another which evokes only the minimum response, elicits a relatively greater response than is warranted. Thus imbalance in favor of the response is commonly engendered when minute stimuli are applied.

It is noted that the term oligodynamic action signifies an entirely different phenomenon than does the term homoligosis. Early microbiologists noted that heavy metals were harmful to bacteria, which were killed by even extremely low concentrations of metals. This is the oligodynamic action of Nageli⁴⁵⁵ which has received ample verification, as is noted by most texts in bacteriology.

Subsequently bacteriologists found that salts of heavy metals⁴⁵⁶ which are toxic in quite dilute solutions are stimulatory when present in concentrations approximately 10-fold more dilute.^{452,457,478,459,460} Growth and fermentation are increased in bacteria and yeasts, by salts of mercury, arsenic (anion), chromium, silver, thorium, platinum, cobalt, manganese, lithium, barium, vanadium, sodium, potassium, zinc, copper, lead, tin, cesium, magnesium, strontium and cadmium. Concentrations as low as 0.000005 molar were effective. Mixtures of salts work as well as single salts, but the effects are not additive.⁴⁵² Richet found that formaldehyde in minute quantities stimulated fermentation in milk,⁴⁶¹ and he was convinced that this behavior was general. Such findings aided acceptance of the Arndt-Schultz rule. Feeney *et al*⁴⁶² suggested that the action is partly a chelation effect.

Following the early work with the toxic heavy metals, Hume,⁴⁶³ Fred,⁴⁶⁵ Hofman,⁴⁶⁶ and Branham⁴⁶⁷ showed that the reaction is indeed general; germicidal compounds were found to give growth stimulation in dilute concentration, as in the following examples (dilution factor is given in parenthesis): ether (100), phenol (350), lysol (500), tincture of iodine (500), alcohol (1000), tricresol (1000), mercurochrome (2000), hexyl-



FIGURE 3-2. EFFECT OF "GERMICIDES" UPON FERMENTATION RATE IN YEAST. DATA OF SCHULZ¹⁰ and BRANHAM.¹¹ The length of the bar indicates the amount of stimulation above the control. The number at the end of the bar gives the dilution at which the stimulation was obtained for each germicide.

resorcinol (8000), metaphen (8000), thymol (30,000), chloramine-T (5000), sodium hypochlorite (5000), formaldehyde (10,000,000) and salvarsan. In general the more potent poisons exhibit this effect at the greatest dilutions.

Sulfonamide stimulated growth of yeast in dilute concentration.⁴⁶⁸ Johnson⁴⁶⁹ suggests that the classical p-aminobenzoic acid-sulfonamide antimetabolite reaction may be explained by this same action, because p-aminobenzoic acid acts in such dilute concentration (the inhibition

ratio is 1/23,000) and because compounds with no structural similarity, such as ethyl carbamate, also counteract sulfonamides. Another compound which resembles p-aminobenzoic acid structure, arsanilic acid, also stimulated the growth of bacteria.^{470,471} Trichothecin stimulated mycelial growth of *Fusarium oxyaporum*.⁴⁷² Pimelic acid⁴⁷³ and indole 3-acetic acid⁴⁷⁴ stimulated growth of microorganisms when present in sub-inhibitory concentrations. In setting up an assay for diethylstilbesterol based upon its antimicrobial action, a consistent growth stimulation was noted just below the inhibition concentration.^{474a}

When Southam and Erlich noted that phenolic type compounds stimulated growth at concentrations below inhibitory levels, they suggested the term *hormesis* for this effect. "The term *hormesis* (adj. hormetic) is proposed to designate such a stimulatory effect of sub-inhibitory concentration of any toxic substance on any organism".⁴⁷⁵ Hormesis is a useful term when designating the action of low levels of antibiotics.

Similar effects were noted with antibiotics such as actinomycin,⁴⁷⁶ gramidicin,⁴⁷⁷ clortetracycline,^{477a} penicillin,^{478,479,480,481,482,483} streptomycin,⁴⁸³ and nystatin,⁴⁸⁴ the reaction being usually run in agar. The antibiotic is often found to be surrounded by a zone on inhibition, and a zone of increased growth within an area of normal growth. The explanations usually offered state that this effect is due to increased food being available to those organisms growing next to the clear zone of inhibition, and that nutrients or other stimulatory material are released when cells undergo lysis under the influence of the drug. The extra food explanation is invalidated by experiments in which different concentrations are obtained in a series of tubes^{480,485,486} instead of by a gradient diffusion through agar, and by experiments illustrating *hormesis in vivo*.^{487,488,489,490,491,492} Both explanations are invalidated by the beautiful experiment of Garrod⁴⁹³ in which the organisms were diluted to give isolated colonies (Fig. 3-3).

Oxidation,⁴⁸⁴ glycolysis⁴⁸⁴ and respiration⁴⁹⁴ has been enhanced by low levels of antibiotics. Another result which cannot be explained easily as a simple nutrient effect is the fact that prior exposure to low levels of penicillin increases the resistance of *E. coli* to serum. The data form a diphasic curve with two separate peaks of increased resistance at different low levels of penicillin.⁴⁹⁵ Decomposition products of chloramphenicol also increased the growth of *E. coli* above that seen in control tubes.⁴⁹⁶ McElroy⁴⁹⁷ has reviewed the increased respiration in yeasts, bacteria, sea urchin eggs and frog muscle produced by such compounds as substituted phenols, phenylurethane, chloral hydrate, chloretone, dinitrophenol and phenobarbital.

Examples of the stimulation of plants or plant parts are presented

earlier in this chapter under mode of action. More examples may be gleaned from the bibliography of Chapter 7, while others are given here. Carrot tissue grew faster in the presence of a small quantity of dihydrostreptomycin.⁴⁹⁸ Seedlings were stimulated to grow faster in the presence of a variety of antibiotics.⁴⁹⁹ When a crystal of chlortetracycline was placed on an agar plate containing algae, there was a ring of stimulation of the growth at the periphery of the circle of inhibition, much as is seen in bacterial experiments, and the plant grew to a greater depth in this

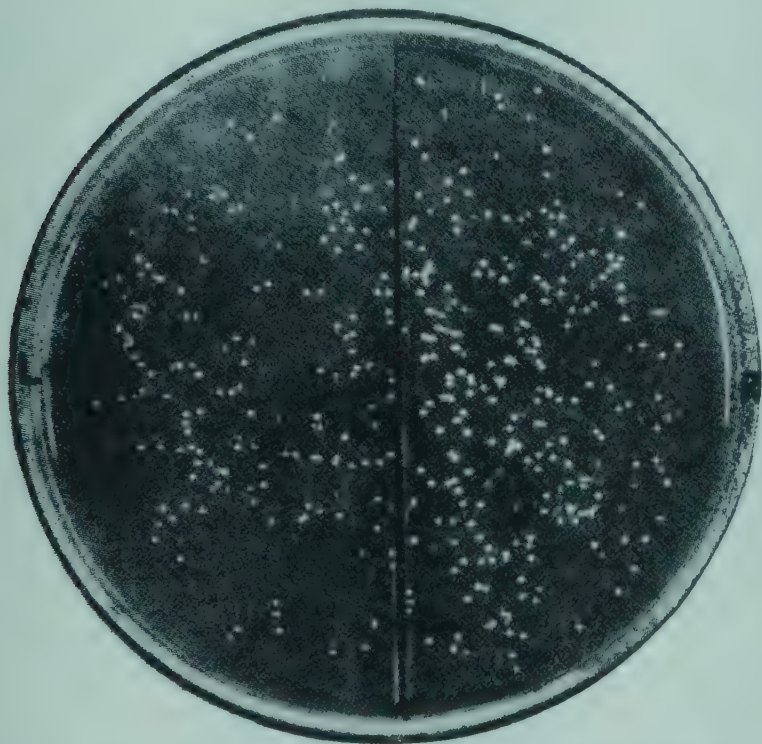


FIGURE 3-3. ANTIBIOTIC GROWTH STIMULATION IN ISOLATED COLONIES.⁴⁹³ Divided agar plate culture of *Staph. aureus* (Oxford "H" strain, of normal penicillin sensitivity). Medium in the right half contains 0.004 unit of penicillin per ml. Incubated at 35° C. for 17 hours.

zone of stimulation.⁴⁶² Antibiotics also increased the photosynthesis and sucrose storage in algae⁵⁰¹ and growth in wheat roots.^{499,501} Maximov⁵⁰¹ reported that weak doses of even the most poisonous substances stimulated plant growth, and cited ethanol at a dilution of 0.003–0.007 molar and phenol at 0.0001 molar as examples. Disodium arsenate stimulated⁵⁰² such plants as wheat, peas, potatoes, beans and radishes at 25–75 ppm DDT^{504,503} and other insecticides⁵⁰⁵ stimulated plant growth, blooming and vigor, and increased the yield of potatoes independently of any insecticidal effect. A syngergism was found to exist between indo-

leacetic acid and antibiotics.⁴³⁴ Nickell²⁹⁶ reported that the antipurine analog, 2,6-diaminopurine inhibited the growth of all plants tissues tested, but that at 0.1 ppm it stimulated growth (about 25%) of sweet clover callus. Chelating agents stimulated plant growth,⁵⁰⁶ and the action of gibberellin (produced by a "parasitic pathogen") is classic.⁵⁰⁷ Other growth promoting substances, including indoleacetic acid, are evidently produced by parasitic plant pathogens, and may account for some of the growth seen in such diseases as crown gall, smuts and rusts.⁵⁰⁷

Bacitracin enhanced the growth of a protozoan.⁵⁰⁸ Evidently little work has been done with low levels of antibiotics in tissue culture. Firket *et al*⁵⁰⁹ found that a low concentration of pantotheine stimulated growth in chick embryo fibroblasts. Oxytetracycline and tetracycline stimulated growth in tissue culture.⁵¹⁰

Some work has been reported in cell-free or cell-particulate systems, but one suspects that, as Nicholl indicated for the plant field, few experiments have been attempted using sufficiently low concentrations of antibiotics. Hardin and Young⁵¹¹ found that arsenates and arsenites increased carbon dioxide production in yeast juice. Low levels of chloramphenicol stimulated bacterial esterase activity, and high levels retarded this enzyme markedly.⁵¹² In liver mitochondria, the stimulation was noted, while the retardation was not noted at the same concentration. It is fitting to speak of enzymes because some of the effects observed may eventually be explained in terms of enzyme kinetics, enzyme production and the regulation of the balance between enzyme inhibition, enzyme products, enzyme substrates and cell stimulators. However, enzyme systems should not be expected to display the full range of regulatory phenomena seen in cells. The action of hormologants must be a part of the regulation of life processes.

Antibiotics act by preventing and/or reducing infections in animals. Treated young animals grow faster simply because the energy and metabolic processes intended for growth are not "wasted" in combating the disease. Antibiotics apparently act more subtly by reducing the micro-invasions in the mucosal linings of gastrointestinal tract (and lung), by increasing the effectiveness of body defenses (such as phagocytosis) and by reducing the relative amount of morphologic (and metabolic) elements of absorptive areas which are devoted to defense (or post-invasive reconstruction). Antibiotics may act via intestinal synthesis. Antibiotics act directly upon the cells and tissues of the host. This hormetic action can be seen as a part of a general phenomenon. The action is consistently noted when the animals are living under suboptimum conditions.

It is evident that antibiotics are not unique in stimulating the growth of animals, since antibiotic derivatives, inactivated antibiotics, many

surfactants, arsonic acids and even minerals give a similar action. It is equally evident that this phenomenon is not restricted to animals, but is seen in plants, and has been studied superficially by many investigators in microorganisms. Other types of stimuli produce a similar reaction.

Although the effects of low levels of radiation have not been studied extensively, enough data are available to suggest confirmation of the generalized theory. Taliaferro and Taliaferro⁵¹³ review the effect of low doses of x-rays on immunity. Although the results are not always consistent, several investigators report a beneficial effect, such as increased survival time in rabbits subjected to diphtheria toxin,^{514, 515} increased agglutinins and hemolysin in guinea pigs,⁵¹⁶ increased agglutinins in mice⁵¹⁷ and humans,⁵¹⁸ and increased hemagglutinins in dogs.⁵¹⁹ Strong irradiation has no such effect and usually causes no effect or decreases antibody formation. Other beneficial effects of x-rays are longer survival of rabbits injected with streptococci, increased rate of healing in local abscesses in rabbits, improved general combat of experimental pneumonia in guinea pigs and dogs, in cholera-infected guinea pigs and in inflammation in humans⁵¹³ (particularly for skin lesions). Growth of plants,^{520, 521, 522} paramecia,⁵²³ and bacterial or fungal respiration,⁵²⁴ growth,^{525, 526, 527, 528} and fermentation,^{529, 530, 531} are stimulated by low doses of x-rays, radiations from radium, or ultraviolet light. Mild x-ray treatment increases B.M.R. of rats.⁵³² Lea⁵³³ indicates the general nature of the growth stimulation by small doses of radiation, while modern reviews treat the subject with skepticism. Little recent work has been reported using minute doses.

Small doses of fast neutrons stimulate the growth of primary roots and accelerate emergence of lateral roots in corn.⁵³⁴ Lorenz *et al*⁵³⁵ report that a low level of γ -rays (0.11 r/day) increased the survival time in mice. Quite surprisingly, to those who have not contemplated hormoligosis, the weights of the mice treated with minute doses of radiation were heavier than unirradiated controls. The same phenomenon was seen in guinea pigs and rabbits. The authors suggest that "a mechanism might exist that overcompensates for low-grade destructive effects by simulating stimulation." More recently the life span of the flour beetle has been shown to be lengthened by a single relatively low dose, or smaller daily doses of γ -rays.^{535a}

Other stress agents such as an atmosphere of very dilute ether or small injuries stimulate growth in plant seedlings.⁵³⁶ Ethylene, acetone, propylene or carbon monoxide induce rooting in plant stems, and ethylene also produces earlier flowering, and cell division in low concentrations, while it kills plants in higher concentrations.⁵³⁷

Cold shock modifies the embryo of many plants to overcome dormancy

and increase vigor.⁵³⁷ Cold may also increase the nutation rate,⁵³⁸ and thus probably the growth rate of some individuals under adverse conditions. A weak electric current causes increased growth of tissues on one side of the stem or root.⁵³⁹ This may be due to increased auxin on one side, or it may be related to the accelerated streaming.⁵⁴⁰ Handling laboratory rats increases their growth rate.⁵⁴¹

It is apparent that at least the hormesis part of hormoligosis may be accepted as a general law of nature. Speculation on a possible explanation of the phenomenon may stimulate work to elucidate the basis of the reaction.

G. SPECULATION

It has been noted that antibiotics act as biological stabilizers in animals; they exert their greatest effect when presented to animals under conditions of mild stress, such as: the shock of leaving the relatively constant and ideal prenatal environment for the less favorable outside environment; the condition of the semi-starved birth runt; the bacteriologically dirty environment; the excessive production of eggs; the existance of poor weather conditions; the marginal and/or deficient dietary regimes; and of course, the presence of infectious disease. Under such conditions the growth rate is regularly depressed, and the feeding of hormoligants more consistently increases the rate of growth. How this action occurs is, at present at least, in the field of mere speculation.

Any stimulus to a cell changes (disrupts) the physical-chemical structure of the system. The stimulus may be transmitted (change the structure) to all parts of that cell or to other cells, either as a part of the stimulus or as a part of the response. Part of the response may be a relatively specific action dictated by the stimulus: a more constant and general part of the response, as indicated by C. Bernard, is a reconstruction of the changed parts in order that the cell may continue its living equilibrium between stimulus and response. Evidently, the rebuilding cell molds itself more in keeping with the current environment. It becomes better than before—at least during the reconstruction period. This represents a part of the defense mechanism of the cell. Lillie⁵⁴² states that the stimulus is always accompanied by variations in electrical potential and permeability of the cell membrane. He gives adequate evidence to demonstrate the increased permeability resulting from many types of stimulation. This alone could account for many of the observations recorded on the effect of antibiotics.

Since biocatalysis is normally considered in terms of hormones and enzymes as well as in terms of inorganic reactions, of chelation and of reduction in surface tension, these processes should have place in an

attack upon the problem. It is logical to suggest that when a minute amount of an antimetabolite is presented to the cell, the resulting action may slightly but effectively reduce the quantity of functioning enzyme to such a low level that the enzyme-producing mechanism of the cell is initiated or accelerated. The net effect may be the presence of more functioning enzyme in the system after the antimetabolite is added than before: and a consequent acceleration of metabolic reactions involved (if the enzyme was the limiting factor).

Another suggestion⁵⁴³ is that the hormoligant is received by the cells as a signal that another stress is about to descend upon it. Since the categories of information a cell can receive are probably rather limited, a variety of stimulants would be received in a somewhat general manner and a general response would be expected. The response may be a defensive reaction, to prepare metabolic, energetic and enzymatic processes for "battle." When the expected stressor comes in no greater quantity than the least stimulant, the massed cellular resources are expended in more effective processes of anabolism and growth. McElroy⁴⁹⁷ and Lamanna⁵⁴⁴ suggest that one process may be stimulated at the expense of another. Thus respiration may be stimulated by a variety of compounds which inhibit cell division, luminescence or assimilation.

Some compounds may be changed into metabolically useful or different compounds which are themselves stimulants. Smith⁵⁴⁵ found that bacterial decomposition products of chloramphenicol stimulate growth of *E. coli*.

Another possibility⁴⁵⁴ involves the balance of stimulus and response at a cellular, tissue or organismic level. As all morphological and physiological processes have chemical bases, so an explanation in terms of chemistry or of a stimulation and response mechanism might be found. Philosophic considerations suggest that the minimum response of a cell must be a unit response for any single action. How many molecules constitute the unit response? If only one enzyme is produced as the minimum response, the rate of a reaction may increase only 5–10% if there are 10 or 20 other molecules of the same enzyme already working in the cell, but it may increase the rate of reaction 20 to 2,000,000 times (depending upon the turnover number of the enzyme) if it is the first molecule of that particular enzyme to be produced in the cell. More could be said only if one knew the number of molecules produced by a cell, as it starts to make a given enzyme. Presumably a different number would be formed by adaptive enzyme formation than in constitutive enzyme formation. Nor can one form any notion of how many enzyme systems are affected directly.

Assume that one or many (10, for example) units of minimum stimuli

elicit only the single unit response, and that 5 units of stimuli (detrimental) are exactly counteracted by one unit response—then if an organism is given 5 stimuli units, no net response would be visible as growth, respiration or otherwise. One to four stimulus units would elicit the minimum response which is greater than would be needed to counteract the stimulus, and the result is a net response. More than 5 stimuli units may produce a net depression, unless the added stimulus evoked the second response unit. Apparently nature has difficulty in balancing the response to the stimulus only near the minimum response. At any rate, only such a simple explanation as this can be expected to account for such a basic and broad reaction.

In this way there is posed the problem of the minimum response. Examination of this question is one of the basic research paths opened by the consideration of antibiotics in nutrition.

H. CONCLUSION

It may be concluded that the growth stimulation of animals by dietary antibiotics is complex. Part of the reaction appears to be “simple”—the prevention or alleviation of infectious disease. A second part may be one or more of a number of reactions, such as intestinal synthesis, increased appetite, adaptation response, etc. A third part of the growth stimulating action appears to be hormoligosis. The net response seen in the animals is the combined response from all of the factors (Fig. 3-4).

The general presentation of the hormetic aspects of hormology illustrates that antibiotics act as do other toxins in producing stimulation at sub-inhibitory concentrations. This aspect is easily missed in animal work for two reasons. Experiments are not generally planned to cover such a wide range as is needed to obtain both the stimulus and the inhibition, therefore both effects are rarely studied in detail. Secondly, animal experiments are often complicated by other factors. Each drug would have a slightly different response curve, and this would be expected to vary with other conditions. The whole curve (Fig. 3-4, dashed line) may be shifted to the left in germfree animals (seen with both ether and barbiturates). A given quantity of hormoligant would be expected to yield different results when species or conditions are changed.

Under practical conditons these effects (and probably others) coalesce to give a single overall curve. The germfree experiments should eliminate some of the effects to show that part of the picture which represents a direct action upon the animal. A suggestion of the overall view is given in Figure 3-4, using oxytetracycline in chicks as an example. As more is learned about the reactions of stimulus and response, several other effects

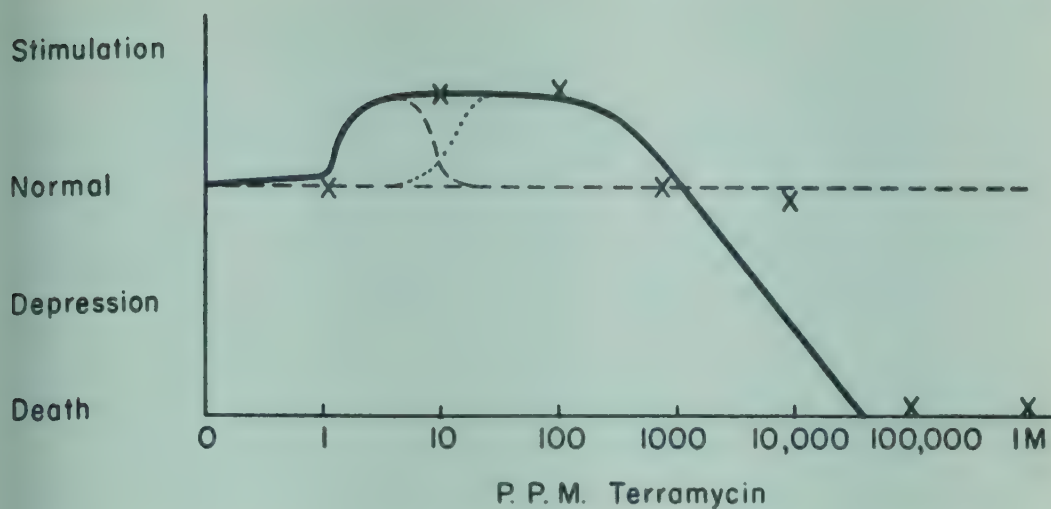


FIGURE 3-4. COMPLEX REACTION USUALLY SEEN AS ONE. The overall curve noted experimentally may be a complex of many factors—two are given here as examples.

discussed will probably appear as contributing factors in growth stimulation.

The general thesis suggests that many more compounds will be found to give a stimulus when administered in the proper sub-inhibitory concentrations. It is hoped this overall view will stimulate effective research on the mechanism of biological response to a hormoligant.

I. REFERENCES

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2. Kidder, G. W. and Dewey, V. C., 1945, Studies on the biochemistry of *Tetrahymena*: I. Amino acid requirements. *Arch. Biochem.* **6**, 425-432.
Serine and glycine were both stimulatory to the growth of this organism while neither was essential for survival.
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Factor "S" or certain unnatural amino acids apparently released a block in the biosynthesis of thiamine imposed by a naturally occurring substance.

4. Luckey, T. D., Briggs, G. M. and Elvehjem, C. A., 1944, The use of *Streptococcus lactis* R for the measurement of folic acid. *J. Biol. Chem.* **153**, 157.

Thymine increased growth in this organism.

5. Skeggs, H. R. and Wright, L. D., 1944, The use of *Lactobacillus arabinosis* in the microbiological determination of pantothenic acid. *J. Biol. Chem.* **156**, 21.

Stimulating substances in natural material are equivalent to oleic acid.

6. Peterson, W. H. and Peterson, M. S., 1945, Relation of bacteria to vitamins and other growth factors. *Bact. Rev.* **9**, 49-109.

Factors which stimulate the growth of bacteria include purines, pyrimidines, streptogenin, ergosterol, oleic acid, many of the B vitamins and many unknown factors.

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A number of substances were found capable of serving as the carbohydrate component of the "rice factor" for chicks.

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Ascorbic acid consistently stimulated the growth of chicks fed "complete purified rations." This growth stimulant may act: (1) as an essential metabolite which is not synthesized at optimum rate; (2) indirectly since it and p-aminobenzoic acid do not give additive effects; or (3) as a detoxifying agent.

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"Sulfasuxidine and streptomycin singly or in combination lead to increased growth responses in chicks receiving our basal diet supplemented with adequate amounts of folic acid."

Diet supplement	Avg. weight (4 weeks)	Total bacterial count
none	155	100×10^6
500γ% folic acid	220	25×10^9
plus 1% succinylsulfathiazole	280	50×10^9
500γ% folic acid plus 10,000 units streptomycin	240	1×10^9
500γ% folic acid plus 50,000 units streptomycin	300	1×10^9

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Chlortetracycline fed chicks grew faster than controls.

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25. Bird, H. R., 1950, New trends in poultry feeding. *Feed Bag* **26**, 15, 59-60.

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Penicillin and chlortetracycline gave increased growth rate in chicks.

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28. Jester, W. R., 1956, The antibiotic regulations for medicated feeds. Symposium for Medicated Feeds by H. Welch and F. Marti-Ibanez. Medical Encyclopedia, Inc., New York, pp. 50-52.

29. Davis, R. L. and Briggs, G. M., 1951, Studies with antibiotics in chick and poult starting rations. *Poultry Sci.* **30**, 767-771.

Streptomycin was not as active in stimulating growth as the other antibiotics tested. Equivalent effects were obtained from the addition of procaine penicillin G, bacitracin, aureomycin hydrochloride, or terramycin.

A combination of 12.5 mg each of aureomycin and streptomycin or procaine G plus bacitracin gave no greater growth stimulation than either of the antibiotics alone. Feed efficiency was improved on all the antibiotic supplemented rations.

30. Williams, O. M. and Hill, J. E., 1952, Effect of antibiotics on the growth of two varieties of turkey poults. *Poultry Sci.* **31**, 769-772.

With the white poults aureomycin plus penicillin gave a significant increase over aureomycin alone. Birds fed the penicillin-aureomycin combination were 48 gm heavier at the end of the experiment than those fed the penicillin alone.

With the Bronze poults, aureomycin and penicillin combined was significantly better than penicillin alone. There was no difference between the combination when compared with aureomycin alone. Streptomycin elicited a growth response above that of the controls with the white poults. The triple combination of antibiotics was no better than the penicillin and/or aureomycin.

31. Haines, C. E., Wallace, H. D. and Koger, M., 1957, The value of soybean oil meal, low gossypol (degossypolized) solvent processed cottonseed meal, low gossypol expeller processed cottonseed meal, and various blends thereof in the ration of growing fattening swine. *J. Animal Sci.* **16**, 12-19.

The feeding of erythromycin (2.5 mg/lb of feed) in combination with procaine penicillin (2.5 mg/lb of feed) as an antibiotic fortification produced faster gains with all nine rations than did the feeding of procaine penicillin 2.5 mg/lb of feed alone.

32. Lillie, R. J. and Bird, H. R., 1953, Evaluation of antibiotic feed supplements and crystalline antibiotics in chick rations. *Poultry Sci.* **32**, 531-535.

Evaluation of crystalline antibiotics found Chloromycetin ineffective; however, Chloromycetin mycelial meal produced a slight growth stimulation. Tomatidine exerted no growth promoting effect. Fumagillin proved toxic at the levels used.

Tyrothricin, gramicidin and neomycin showed evidence of growth-stimulating effect, but their effects were less consistent and of lesser magnitude than those produced by the antibiotics now being used commercially in feeds.

33. McGinnis J. and Jensen, L. S., 1958, A difference in growth response of chicks and turkeys to different antibiotics. *Fed. Proc.* **17**, 484.

Much used antibiotics fail to give a response while new ones, erythromycin and oleandomycin, promote faster growth.

34. Taylor, J. H. and Rowell, 1957, The effect of various levels of penicillin and chlortetracycline in the diet of fattening pigs. *Brit. J. Nutr.* **11**, 111-116.

35. Robertstad, G. W., Sullivan, R., Tucker, J. O. and Glenn, M. W., 1955, Subcutaneous implantation of antibiotics in pellet form to stimulate the growth range lambs and range calves. *Vet. Med.* **50**, 142-143.

None of the following subcutaneous implantations of antibiotics in pellet form significantly increased the growth rate of lambs and calves raised under range conditions: (1) calves—4,000 units of bacitracin within 12 hours of parturition; (2) lambs—6,000 units of bacitracin at 1 to 3 weeks of age; (3) lambs—3,000 units of bacitracin and 4,000 units of neomycin at 1 to 3 weeks of age; (4) lambs—35,000 units of bacitracin and 25,000 units of penicillin.

36. Branion, H. D., Hill, D. C. and Motzok, I., 1952, Effect of subcutaneous implantation of bacitracin on the growth of chicks. *Poultry Sci.* **31**, 1096-1098.

Neither growth nor feed efficiency to six weeks of age were affected by the subcutaneous implantation of a pellet containing 1,000 units of bacitracin in the neck of newly hatched chicks. No undesirable physiological effects were observed as a result of the implantation.

37. Hvidsten, H. and Grotli, B., 1956, Experiments in subcutaneous implantation of antibiotics in pellet form on suckling pigs. *Acta Agric. Scand.* **6**, 138-140. (*Chem. Abstr.* **51**, 5229, April 10, 1957.)

Bacitracin with and without penicillin was implanted as pellets (1 or 2 pellets containing 500 units of bacitracin and 5,000 units of penicillin or 1,000 units of bacitracin) subcutaneously behind the ears of 6 pigs litters (52 animals) 1 to 4 days after birth. In most cases, another implantation was given at 3 weeks. The animals remained with their sows. During the 43rd to 51st day of the experimental period the antibiotic had no effect on weight gain or the health of the animals.

38. Noland, P. R., Tucker, D. L. and Stephenson, E. L., 1952, Subcutaneous implantation of bacitracin in pellet form to stimulate growth of suckling pigs. Rpt. Series 34, Agric. Exper. Sta., Univ. of Arkansas College of Agric.

The subcutaneous implantation of a single 1,000 unit bacitracin pellet

at the base of the ear of 56 pigs, 2 to 5 days of age, produced an 11.3% increase in weaning weight over the controls at 56 days. Forty-four pigs received two pellets and showed a 4.2% increase in weight. A 5.1% weight increase was obtained in 48 pigs receiving four of the pellets.

39. Marshall, S. P., Wing, J. M. and Arnold, T. P. D., 1957, Effects of feeding aureomycin to dairy calves. *J. Dairy Sci.* **40**, 1242-1249.

Following removal of aureomycin from the diet of six calves at 61 days of age, the average body weight gain and hay consumption were significantly lower during the ensuing 30 days than for the six animals previously fed the unsupplemented ration. The effects of its removal from the diet at 121 days of age were variable.

After aureomycin was removed from the diet, the responses of individual animals varied widely, ranging from cases in which gain rate, feed intake and appearance were excellent, to some cases in which there were anorexia, reduced growth, excessive lachrymation and development of diarrhea, rough coat and thickened, scaly hide. Some animals with mild symptoms recovered within a period of a few days to a few weeks and some severe cases had not recovered after 50 to 90 days.

40. West, J. W., 1956, Effect of 3-nitro-4-hydroxyphenylarsonic acid and certain antibiotics in broiler rations. *Poultry Sci.* **35**, 835-842.

The greatest stimulating effect upon growth and feed efficiency was observed when the arsonic compound (45 gm/ton) was added to the basal diet containing no antibiotic, the percentage increase being on the order of 8%.

Definite and rather consistent improvements were noted when the arsonic compound was added to rations containing "low" levels of antibiotics (10 gm/ton); the percentage increases in growth and feed efficiency were of the order of 6% and 4% respectively. The least stimulating effect was observed when the arsonic acid derivative was added to diets containing "high" levels of antibiotics (100 gm/ton), the percentage increase being of the order of 2%.

41. Conrad, J. H. and Beeson, W. M., 1957, Effect of various antibiotic combinations and other bactericidal agents on growing-finishing swine. *J. Animal Sci.* **16**, 1078.

On concrete, six groups of 12 pigs each were full-fed a basal ration of ground corn and supplement mixed to contain 16% protein to 100 lb. then 14% protein thereafter. The treatments, daily gains (lb) and feed per 100 lb of gain (lb) were: control, 1.42 and 327; chlortetracycline 20 gm/ton, 1.46 and 322; chlortetracycline, 20 gm + sodium metaphosphate 60 gm/ton, 1.44 and 322; arsanilic acid 90 gm/ton, 1.54 and 311; chlortetracycline 10 gm + arsanilic acid 45 gm/ton, 1.47 and 323; hygromycin 12 million units/ton, 1.54 and 306.

42. Hanson, L. E. and Ferrin, E. F., 1956, Effect of antibacterial agents on the growth of suckling pigs. *J. Animal Sci.* **15**, 376-391.

In the final experiment in this series, chlortetracycline and procaine penicillin at levels of 5 mg per pound of feed and arsanilic acid at a level of 30 mg per pound of feed produced statistically significant increases in weaning weights of the pigs in one trial. In a second trial, effects on weaning weights were not significant. There was no significant difference between responses to the three drugs.

43. Heaney, D. P. and Thomas, O. O., 1956, The effect of adding stilbestrol and aureomycin to a high-barley fattening ration for steers. Agric. Exper. Sta., Mont. State College, Mimeo. Circular 95. pp. 1-12.

The combination of stilbestrol and chlortetracycline was not as good as either separately.

44. Beeson, W. M., Perry, T. W., Mohler, M., Andrews, F. N. and Stob, M., 1957, Combination of an antibiotic and a female hormone for fattening steers. *J. Animal Sci.* **16**, 845-849.

When hexestrol and chlortetracycline (Aureomycin) (100 mg daily), were fed in combination, the growth stimulatory effect was almost exactly equal to the sum of effects of the two when fed separately, indicating the growth stimulatory effects of these two substances are exerted independently and are additive.

Efficiency of feed conversion was improved when either the hormone or antibiotic was fed alone, but the efficiency of conversion was increased when the two were fed together by an amount that is 1.6 times as great as the sum of the figures for improvement brought about by feeding either alone.

45. Chapman, H. L., Jr., Kidder, R. W., Palmer, A. Z. and Emerson, J., 1957, Effect of level of feed intake on steers fed chlortetracycline and/or diethylstilbestrol on pasture and in drylot. *J. Animal Sci.* **16**, 1035.

A combination of antibiotic and stilbestrol increased gains except for limited-fed steers on pasture. Stilbestrol implants did not increase average daily gain of steers full-fed on pasture as compared to those receiving the basal ration. Average daily gain was higher for cattle fattened on pasture than in drylot, one each level of feed intake.

46. Adams, C. R., Reynolds, W. M., Sherman, W. C. and Luther, H. G., 1955. Diethylstilbestrol and oxytetracycline (Terramycin) in combination for growth promotion in feeder cattle. *J. Animal Sci.* **14**, 1242.

Four groups, each of 20 steers averaging 743 lbs, were supplemented as follows: none, oxytetracycline (TM) 10 gm, per ton, stilbestrol 10 mg per day, and stilbestrol plus TM. Results in each group in order named were: average daily gains, 2.25, 2.47, 2.47 and 2.94 lbs; average pound feed per pound gain, 11.3, 10.8, 9.8 and 9.1; average carcass grade, 9.95, 9.55, 9.1 and 9.55 (Scale: 8 = medium good; 9 = high good; 10 = low choice).

47. Beeson, W. M., Perry, T. W., Mohler, M., Andrews, F. N. and Stob, M., 1956, The effect of feeding a female hormone and an antibiotic alone and in combination to fattening steers. Purdue Univ. Agric. Exper. Sta. Mimeo. A. H. 166.

When both hexestrol and Aureomycin were fed, the increase in growth rate reached 37% which is almost exactly equal to the sum of effect of the two supplements when fed separately, indicating that the growth stimulatory effects of the two substances are exerted independently and are additive. The hormone supplement improved the efficiency for feed conversion by 16%, the antibiotic by 10%. When both the hormone and the antibiotic were fed together, the feed efficiency was improved 26% over that of the controls, thus exactly the sum of the effects of the hormone and the antibiotics fed alone. There was no significant differences in carcass grades among the four lots.

48. Perry, T. W., Beeson, W. M., Andrews, F. N., Stob, M. and Mohler, M. T., 1958, The comparative effectiveness of oral and subcutaneous implantation of diethylstilbestrol in combination with chlortetracycline. *J. Animal Sci.* **17**, 164-170.

The results were additive—5% greater growth with 80 mg CTC per day to calves.

49. Perry, T. W., Beeson, W. M., Mohler, M. T., Stob, M. and Andrews, F. N., 1956, Oral Stilbestrol vs. levels of implanted stilbestrol with and without Aureomycin. Purdue Univ. Agric. Exper. Sta. Mimeo. A. H. 165.

Stilbestrol implantation gave by far better results than feeding the hormone. The most satisfactory hormone level was 36 mg. Feeding 80 mg Aureomycin per day, with any of the treatments, had no growth-stimulating effect except when it was fed with oral stilbestrol. Antibiotics have consistently stimulated the growth rate of cattle when fed with high roughage rations. When fed with high-energy rations, however, the effect of antibiotics has not been consistent.

50. Harris, B., Jr. and Rusoff, L. L., 1956, Effect of chlortetracycline and diethylstilbestrol on growth, carcass and endocrine glands of dairy calves when fed singly and in combination. *J. Dairy Sci.* **39**, 929.

Preliminary results at 16 weeks of age indicate that a growth response was obtained in the groups receiving chlortetracycline. The supplementation of the diet with diethylstilbestrol appeared to have no effect on growth. Only a few cases of scours occurred in this experiment.

51. Rohlf, J. A., 1955, A stilbestrol-antibiotic supplement. *Farm Journal* July, pp. 32, 92.

Steers fed oxytetracycline with stilbestrol gained 13% faster on 6% less feed than steers fed only the stilbestrol.

Lambs fed 10 gm/ton oxytetracycline grew 20-30% faster when stilbestrol was fed at 3 mg/day each.

52. Hentges, J. F., Jr., Black, J. A., Tucker, C. A. and Cunha, T. F., 1955, The effect of chlortetracycline (aureomycin) and diethylstilbestrol on growth and carcass measurements of steers. *J. Animal Sci.* **14**, 1207.

No advantage in steers to combination of chlortetracycline and stilbestrol.

53. Lucas, I. A. M. and Calder, A. F. C., 1957, Antibiotics and a high level of copper sulfate in rations for growing bacon pigs. *J. Agric. Sci.* **49**, 184-199.

In experiments 1 and 2 the best overall rates of growth and efficiencies of food conversion were those of pigs fed from weaning to bacon weight on diets supplemented with both CuSO_4 and penicillin. Supplements of penicillin did not improve growth performance as much as supplements of CuSO_4 . In experiment 3, there was an interaction effect, whereby the addition of Aurofac 2A to diets already containing 0.1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ improved rate and efficiency of growth when the diets included $7\frac{1}{2}$ or $2\frac{1}{2}$ % whitefish meal, but not when they contained no fish meal. This was not confirmed in experiment 4 when the additions of Aurofac 2A improved the performances on all diets. Experiments 1, 3 and 4 showed that during the growing period the addition of antibiotic to a ration already containing 0.1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ is worthwhile. The bene-

ficial effects of the two supplements are at least additive and there is some indication that there might be an interaction effect.

54. Wacker, A., Heyl, W., Buehl, H. and Holthoff, J., 1956, The action of antibiotics as growth substances in animals. II. Studies with inactivated penicillin and copper sulfate. *Arzneimittel-Forsch.* 6, 712-714. (*Chem. Abstr.* 51, 5938, April 25, 1957.)

Addition of 40 mg cysteine, taurine, or inactivated penicillin per kg diet containing 2% protein results in improved growth of baby chicks. Penicillin is the most effective compound. A combination of penicillin and CuSO_4 added to the diet has the same growth-promoting effect as a diet containing 12% protein.

55. Sherman, W. C., Hale, W. H., Reynolds, W. M. and Luther, H. G., 1957, Nutritional uses of tranquilizers in cattle and lamb rations. *J. Animal Sci.* 16, 1020, (in *Proc. Soc.*).

Growth was enhanced by hydroxyzine in all trials and by Rauwolfia or reserpine in four or five. For lambs implanted with 3 mg stilbestrol and 50 mg oxytetracycline and fed hydroxyzine, 0, 0.24, 1.2, 6 and 30 gm per ton of feed or Rauwolfia, 3 gm. per ton, gains and feed efficiencies were respectively: 0.477, 7.35, 0.473, 7.54; 0.547, 6.98; 0.498, 7.19; 0.485, 7.31; 0.551, 7.16. In a second trial, gains and feed efficiencies on 0 and 2 gm hydroxyzine and 2 gm Rauwolfia were, respectively, 0.415, 9.38; 0.531, 7.35, and 0.436, 8.70. Levels of tranquilizers fed did not visibly sedate the animals.

56. Christensen, J. J., 1956, Mutagenic effects of antibiotics. In *Proc. First International Conference on Antibiotics in Agriculture*. National Academy of Sciences and National Research Council, Washington, pp. 13-78.

Some evidence indicates antibiotics are mutagenic agents.

57. Carlson, C. W., 1957, Antibiotics and reproductive performance of chickens. *Feed Age* 7, 42-43, 45, 83.

Hens that were laying at a relatively low rate or producing eggs with below average hatchability were improved most by antibiotic supplementation. On the basis of this work, low levels of antibiotics (10 gm or less per ton—some recommendations are 25 gm/ton, this is presently being studied) would be recommended continuously, supplemented with high levels of antibiotics (50 to 100 gm/ton) in times of stress. Certain conditions of endemic disease may permit the economical use of high levels continuously.

58. Gordon, W., 1956, First International Conference on Antibiotics in Agriculture. National Academy of Sciences and National Research Council. Washington, pp. 153-160.

Aureomycin increased the growth of foals through one year. Growth response was seen in pigs whether the antibiotic was given by mouth or by injection. He proposes one mode of action of antibiotics is in simple prophylaxis against infection, clinical and subclinical.

59. Luckey, T. D., Gordon, H. A., Wagner, M. and Reyniers, J. A., 1956, Growth of germfree birds fed antibiotics. *Antibiotics and Chemother.* 6, 36-40.

Growth data are reported for experiments in which antibiotics were fed to germfree chicks and turkey poults. Birds were fed a high vitamin,

- semisynthetic diet and the following relatively high levels of antibiotics: 23 mg/kg chloramphenicol, 35 mg/kg bacitracin, 70 mg/kg and 450 mg/kg streptomycin, 50 mg/kg oxytetracycline hydrochloride, and 46 mg/kg procaine penicillin. There was a slight consistent decrease in growth rate with these levels, indicating that lower levels of antibiotics should be fed. When the germfree chicks were fed 25 mg oxytetracycline/kg diet, they appeared to grow at a faster rate than did the control chicks fed no antibiotics. Germfree chicks fed 11 mg/kg procaine penicillin showed possible growth stimulation. Growth rate of germfree turkey poults was generally faster when they were fed procaine penicillin, 46 mg/kg diet.
60. Spector, W. S., 1957, *Handbook of Toxicology*, Vol. II. Antibiotics. W. B. Saunders Company, Philadelphia.
 61. Goldberg, H. S., Read, B. E. and Goodman, R. N., 1958, Studies on the emergence of streptomycin-resistant bacteria as a result of low-level long-term feeding of streptomycin. *Antibiotic Annual 1957-58*, 144-148.
Streptomycin accumulates in the gut when fed continuously.
 62. Welch, H., 1950, Absorption, excretion and distribution of terramycin. *Ann. New York Acad. Sci.* **53**, 253-265.
 63. Paine, T. F., Jr., Collins, H. S. and Finland, M., 1948, Laboratory studies with aureomycin. *Ann. New York Acad. Sci.* **51**, 228-230.
 64. Bush, L. J., Jacobson, M. L. and Hartman, P. A., 1957, Levels of chlorotetracycline in the rumen fluid of dairy calves following oral administration of the antibiotic. *Antibiot. and Chemotherapy* **7**, 9-12.
The antibiotic disappeared rapidly from the rumen fluid following oral administration.
 65. Shidlovsky, B. A., Prigot, A., Maynard, A. L., Felix, A. J. and Hjelt-Harvey, I., 1958, Absorption, diffusion and excretion of studies on the phosphate complex salt of tetracycline. *Antibiotics Annual 1957-58*, 459-468.
Tetracycline phosphate is absorbed more rapidly and antibiotic blood levels are higher than was found with the chloride salt.
 66. Carlozzi, M., 1958, Evaluation of antibiotic blood level enhancement factors. *Antibiot. Med. and Clin. Ther.* **5**, 146-151.
Blood levels of antibiotic were highest when glucosamine was given orally with the antibiotic. Tetracycline was tested in combination with glucosamine, citric acid, Na hexametaphosphate and phosphate complex.
 67. Durbin, C. G., DiLorenzo, J. J., Randall, W. A. and Wilner, J., 1953, Antibiotic concentration and duration in animal tissues and fluids. II. Chicken blood, tissue, and eggs. *Antibiotics Ann. 1953-54*. Proc. Symposium on Antibiotics, Washington, D. C., pp. 428-432.
Chlortetracycline is present in the blood serum of a majority of chickens fed a diet containing 50, 100 or 200 ppm of the drug. The antibiotic can be found in the lean flesh and liver of a considerable proportion (40%) of chickens fed 50 and 100 ppm. chlortetracycline. This activity is lost on cooking. When the concentration of chlortetracycline in the feed is increased from 200 to 20,000 ppm the serum and tissue concentration of the drug tends to rise. After continued feeding the antibiotic concentration tends to decline, especially in the intestines. This is probably due to decreased food intake.

68. Vavich, M. G., Kemmerer, A. R. and Fleming, W., 1955, Unidentified factors in foods and feeds and the physiological availability of vitamins, minerals amino acids and other nutrients. Research Progress, Univ. of Arizona.

Chicks fed Aureomycin were found to deposit Aureomycin in their flesh and eggs. Below a level of 500 gm/ton of feed no antibiotic was deposited in eggs. At the 500 gm level, 0.043 ppm were found in the eggs, at 1,000 gm level, 0.063 ppm and at 2,000 gm level, 0.054 ppm. At the 100 gm level no antibiotic was deposited in the flesh. At 1,000 and 2,000 gm level it was. Individual chickens differed in the amount of antibiotics they could deposit in their eggs or flesh.

69. Hester, H. H., Landagora, F. T. and Rusoff, L. L., 1954, The distribution of aureomycin in the body of dairy calves showing a growth response when the antibiotic is administered orally or intramuscularly. *J. Animal Sci.* **13**, 988-989.

An investigation was made of the absorption, distribution and excretion of aureomycin in the body of calves administered nutritional levels of the antibiotic and showing a growth increase of 19% above the controls at 16 weeks of age. The antibiotic was present in the blood plasma, bile and urine of the Aureomycin-administered calves. The livers and kidneys of the injected calves contained measurable amounts of Aureomycin while a trace was present in the liver and kidney of one calf of the oral-fed group. The spleen, thymus, pituitary and muscle showed no detectable antibiotic in any of the calves. No aureomycin was found in the rumen of the injected calves. This finding is in opposition to the theory that explains the mode of action of Aureomycin through alteration of the rumen microflora.

70. Luther, H. G., Reynolds, W. M., McMahan, K. R. and Kersey, R. C., 1953, Antibiotics carry-over in tissues of livestock. *Antibiotics Annual* **1953-54**, 416.

Very little antibiotic is detected in tissues of poultry and pigs fed 50 gm of oxytetracycline per ton of food. The following values are reported for animals fed 200 gm per ton: for poultry, large intestines had 1.32; liver, .25; kidney, .81; and fecal matter, 23.83 ppm. The swine showed .677 for intestine and .23 for kidney.

71. Anderson, G. W., Epps, N. A., Snyder, E. S. and Slinger, S. J., 1958, Comparative effectiveness of feeding aureomycin and dipping in an aureomycin solution as a means of preserving poultry meat. *Poultry Sci.* **37**, 174-179.

Chlortetracycline was as effective when fed for 5 days at 1,000 ppm as when employed as a dip at 10 ppm in exerting a bacteriostatic effect on microorganisms present in poultry tissue after slaughter.

Lower levels were not tried.

72. Carpenter, L. E. and Larson, N., 1953, Antibiotics and the reproduction of swine. *J. Animal Sci.* **12**, 812-818.

Feeding a high level of aureomycin (2 gm/100 lb of a mixed animal vegetable protein basal ration) to pigs from weaning through two complete gestation and lactation periods had no harmful or beneficial effect on reproduction.

Neither the feeding of 1 gm of aureomycin hydrochloride via capsule

three times a day prior to parturition nor the intramuscular injection of 900,000 or 1,000,000 units of penicillin sodium G just prior to parturition resulted in transfer of the antibiotic across the placental tissues of the sow.

73. Robinson, P., 1952, Controlled trial of aureomycin in premature twins and triplets. *Lancet* **262**, 52.

Increased growth and decreased mortality is seen in the group of premature infants receiving chlortetracycline.

74. Snelling, C. E. and Johnson, R., 1952. The value of aureomycin in prevention of cross infection in the hospital for sick children. *Conn. M. A. J.* **66**, 6.

Lower premature infant mortality was seen when 50 mg of chlortetracycline was administered daily than in the control group given none.

75. Sizemore, J. R., Lillie, R. J., Bird, H. R. and Denton, C. A., 1955, Further studies on the influence of Aureomycin in the chick diet upon subsequent reproductive performance of a laying hen. *Poultry Sci.* **34**, 432-435.

There appeared to be a definite relationship between growing and breeder diets and embryonic mortality during the latter two weeks of the incubation period. In every case, hens whose growing diet contained an antibiotic showed a lower embryonic mortality. Those hens fed crystalline aureomycin in the breeder diet further decreased mortality in embryonic development in three of four comparisons.

76. Ellis, N. R., 1956, Antibiotics in reproduction. First International Conference on Antibiotics in Agriculture. Nat. Acad. Sci. and National Research Council, Washington, pp. 69-72.

The use of the common antibiotics has progressed to the point where we are reasonably certain that there are no more harmful effects in the reproduction cycle than there are in the growth cycle.

77. Stokstad, E. L. R. and Jukes, T. H., 1951, Effect of various levels of vitamin B₁₂ upon growth response produced by aureomycin in chicks. *Proc. Soc. Exptl. Biol. and Med.* **76**, 73-76.

The mortality of vitamin B₁₂ deficient chicks on the diet containing no vitamin B₁₂ was reduced by aureomycin.

78. Harper, J. A. and Babcock, W. E., 1953, The effect of penicillin on early mortality and growth in poults. *Poultry Sci.* **32**, 179-180.

Twenty poults within each feeding treatment were given orally 200 mg. of an aqueous suspension of procaine penicillin when removed from the incubator. Mortality was markedly reduced in all lots where penicillin was included in the starter rations as compared to control lots. Penicillin given orally was not as effective in reducing losses as including penicillin in the feed. In all instances, the inclusion of penicillin in the feed materially increased the growth rate. Poults given penicillin orally and fed diets with or without penicillin were in five of the six comparisons slightly heavier at 2 weeks of age than poults not receiving penicillin orally.

79. Scott, M. L., Holm, E. R. and Reynolds, R. E., 1954, Studies on pheasant nutrition. 3. Effect of antibiotics, arsenicals and thyroactive compounds upon growth and feathering in pheasant chicks. *Poultry Sci.* **33**, 1261-1265.

The results presented in this report show that Aureomycin, bacitracin, penicillin and Terramycin are all effective in increasing the growth rate

in pheasant chicks. This stimulation in growth rate was intermediate between that which has been observed under similar conditions with turkey poults and that obtained with chicks. The pheasants receiving the antibiotics appeared stronger and healthier than those receiving the same diet without the antibiotics. In one experiment in which weak pheasant chicks were used, mortality was reduced by supplementing the diet with an antibiotic. Although feathering was improved in the lots receiving the antibiotics, feather picking was not prevented under the conditions of these experiments.

0. Jukes, T. H., 1955, Antibiotics in Nutrition. Medical Encyclopedia, Inc., New York, pp. 128.

This fine review with over 500 references gives a summary of the proposed mechanisms of the antibiotic growth effect; the effect of feeding antibiotics upon the requirements for vitamins, minerals and protein, the effect of antibiotics in production of poultry, swine, beef, milk and eggs, and the physiologic effect on liver, blood formation and miscellaneous other things.

1. Jolliffe, N., Frontali, G., Maggioni, G., Corbo, S. and Lanciano, O., 1956, Effects of chlortetracycline on weight gain of Italian children ages 6-10 on diets relatively low in animal protein. *Antibiotics Annual* 1955-56, 19-26.

Children in orphanages receiving 20-30% less calories and 14-40% less animal protein than the recommended allowances were divided into two groups.

One group¹⁸¹ received 20 mg of chlortetracycline per day and the control group¹⁵⁷ received placebos. The only significant changes seen were that the children below 20 kg grew faster than the control group.

2. Patrick, H., 1953, Comparative growth promoting value of procaine and diamine penicillin for chick. *Poultry Sci.* 32, 554-555.

Marked increased growth rates were seen in chicks fed 0.5 ppm procaine penicillin when compared to control chicks fed a corn-wheat-raw soybean diet.

3. Reynolds, J. W., Runnels, T. D. and Waller, E. F., 1951, A comparison of terramycin and penicillin at various levels on rate of growth and feed efficiency in broiler diets. *Poultry Sci.* 30, 928.

Broiler chicks housed in batteries and fed a practical broiler mash grew best and had the best feed efficiency when 2 gm procaine penicillin was added to each ton of mash.

4. Waibel, P. E., Abbott, O. J., Baumann, C. A. and Bird, H. R., 1954, Disappearance of the growth response of chicks to antibiotics in an "old" environment. *Poultry Sci.* 33, 1141-1146.

5. Libby, D. A. and Schaible, P. J., 1955, Observation of growth responses to antibiotics and arsonic acids in poultry feed. *Science* 121, 733-734.

The relative growth responses obtained when low levels of antibiotics and arsonic acids were added to poultry feeds were found to decrease progressively over a four year period. The weights of control birds not fed these supplements, however, have been gradually increasing. Both the controls and antibiotic-fed birds increased in weight during the four year period, but the former gained at a much greater rate than the latter. The apparent decreased effectiveness of the growth promotants, therefore, is caused by a relatively greater increase in the rate of growth of birds fed the control ration.

The reduction in the mortality of chicks that we have observed during the last four years adds support to the postulation that a reduced germ load develops.

86. MacGregor, H. I., Blakely, R. M. and Anderson, R. W., 1954, Antibiotics in the diet of turkey poultts of various ages. *Poultry Sci.* **33**, 36-38.

The addition of 4.4 ppm procaine penicillin at 8 weeks of age had no effect on growth rate of those poultts not receiving this supplement from 1 to 8 weeks of age. The addition of penicillin at 8 weeks of age to the diets of poultts which had received penicillin from 1 to 8 weeks of age had no effect on the growth rate of males to 20 weeks of age, but significantly increased the growth rate of females.

87. MacKay, A. M., Riddell, W. H. and Fitzsimmons, R., 1953, Terramycin supplement for dairy calves. *J. Animal Sci.* **12**, 19-23.

Terramycin when fed for 12 weeks at the rate of 30 mg/100 lb body weight to young dairy calves, receiving a liberal ration of milk, calf starter, and good quality hay, produced a significant increase in growth, stimulated the appetite, and improved general appearance when compared to the controls.

88. Berg, L. R., Bearse, G. E., McGinnis, J. and Miller, V. L., 1950, The effect of removing supplemental aureomycin from the ration on the subsequent growth of chicks. *Arch. Biochem.* **29**, 404-407.

The effect of adding and deleting an aureomycin fermentation product from the diet of $4\frac{1}{2}$ week old chicks was studied. Deleting the aureomycin product from the ration resulted in a cessation of the accelerated growth response observed during the first $4\frac{1}{2}$ weeks of life when the aureomycin fermentation product was fed. Adding the aureomycin product to the ration at $4\frac{1}{2}$ weeks of age caused an immediate acceleration of growth.

89. Hill, C. H. and Kelly, J. W., 1953, The effect of antibiotics on the growth of chicks raised in new quarters. *J. Nutr.* **51**, 463-466.
90. McGinnis, J., Stern, J. R., Wileox, R. A. and Carver, J. S., 1951, The effect of different antibiotics on growth of turkey poultts. *Poultry Sci.* **30**, 492-496.

Experiments conducted to determine the comparative effect of different antibiotics on the growth of turkey poultts, showed that penicillin was more effective than terramycin or streptomycin at levels of 5, 10, or 20 mg/kg and stimulated growth equal to that obtained with a combination of 25 mg each of terramycin, aureomycin, streptomycin and penicillin.

91. MacGregor, H. I., Blakely, R. M. and Anderson, R. W., 1952, Growth response of turkey poultts to procaine penicillin added to the diet at various ages. *Poultry Sci.* **31**, 924.

Young turkey poultts fed 8.8 ppm of antibiotic were 60% heavier at 4 weeks than controls and 6% heavier at 20 weeks. Delay of supplementation to 4 weeks voided the effect entirely.

92. Saxena, H. C., Berg, L. R. and McGinnis, J., 1952, Factors affecting the growth response of chicks and turkey poultts to antibiotics. *Poultry Sci.* **31**, 1070-1074.

Diamine penicillin was added at levels of 0.5-3 ppm and terramycin HCl at concentrations of 1-5 ppm. The responses obtained with penicillin and terramycin and with combinations, were more consistent and

greater for chicks kept on litter than for those kept in battery brooders. Diamine penicillin gave the greatest response under both environmental conditions. Combinations of the two antibiotics gave no greater growth response than penicillin alone.

93. Branion, H. D. and Hill, D. C., 1952, Antibiotics and the growth of goslings. *Poultry Sci.* **31**, 1100-1102.

Heavier birds were obtained at 8 weeks when antibiotics were added to the ration. Food efficiency was also increased.

94. Slinger, S. J., Snyder, E. S. and Pepper, W. F., 1953, Effect of penicillin on the growth of goslings. *Poultry Sci.* **32**, 396-400.

Penicillin caused growth increases in the groups fed pellets or pellets and grain. These increases were significant in males at 2 weeks of age, but not at 4, 6 or 8 weeks of age. The weight increases in females were not significant. When fresh lawn clippings were before the birds at all times, penicillin caused no increase in weight. Feed efficiency was not improved by penicillin. The results indicate that a growth response to penicillin was obtained in the early stages of growth in the absence of fresh green feed. No such response was obtained when fresh green feed was available even in contaminated premises.

95. Mraz, R. F., Boucher, R. V. and Callenbach, E. W., 1956, The response of bobwhite quail to antibiotics. *Poultry Sci.* **35**, 76-80.

Growth response to antibiotic supplementation was variable. Aureomycin, bacitracin, penicillin and terramycin were significantly effective in the first experiment, with no significant differences occurring among them at 3 weeks of age. In the second experiment in which neither aureomycin nor streptomycin was fed, a significantly better growth response occurred with bacitracin and penicillin than with terramycin.

Streptomycin at a level equivalent to 10 gm of free base per ton of diet had no effect on rate of growth.

96. Scott, M. L., Holm, E. R. and Reynolds, R. E., 1954, Studies on pheasant nutrition. Effect of antibiotics, arsenicals, and thyroactive compounds upon growth and feathering in pheasant chicks. *Poultry Sci.* **33**, 1261-1265.

97. Amschler, J. W. and Pammer, H., 1955, Entenmateversuch mit Bi-Con TM (Terramycin) (Duck fattening experiment with Terramycin). *Bodenkultur* **8**, 327-330. (*Nutrition Abstr. and Rev.* **26**, 3944, 835.

The ducklings given terramycin and vitamin B₁₂ made better weight gains, especially in the first three weeks: over the whole period 7.1% better, with less feed. The overall improvement in feed efficiency assessed by Lehmann's method was 7.7%. Losses were fewer. The flesh of the birds was pure white and of excellent quality.

98. Nowak, H., 1956, Entenmast mit Terramycin and Vigofac (Fattening ducks with Terramycin and Vigofac). *Arch. Geflüge Ikde* **20**, 35-40.

	Weight gains	Eight week results	
		Feed efficiency	
Controls	2,112 gm 100%	266	100%
Terramycin group	2,232 gm 105.7%	246	92.5%
Vigofac R group	2,257 m 106.9%	244	91.7%
Terramycin-Vigofac R group	2,342 gm 110.9%	239	89.8%

99. White-Stevens, R., Zeibel, H. G. and Walker, N. E., 1956, The use of chlortetracycline-aureomycin in poultry production. *Cereal Sci.* 1, 101-108.

Continuous or prophylactic feeding of aureomycin at levels of 50-200 gm/ton of total diet produced significant increases in growth rate, livability, meat yield, quality and feed conversion among growing chickens, turkeys, and ducks, and improved egg production and feed-to-egg ratios in laying and breeding fowls and turkeys. These advantages were specially noted under conditions of endemic diseases of an acute and/or a chronic etiology.

100. Branion, H. D., Anderson, G. W. and Hill, D. C., 1953, Antibiotics and the growth of ducks (with a review of possible mechanisms by which antibiotics stimulate growth). *Poultry Sci.* 32, 335-347.

The addition of aureomycin, penicillin and Terramycin at levels of 10, 25 and 100 ppm and streptomycin at 25 ppm to a mixed animal-vegetable protein ration had no effect upon the growth or feed efficiency of ducks fed these supplements to 6 weeks of age.

101. Ferrando, R. and Daumin, A., 1956, Les Antibiotiques dans l'alimentation du canard (Antibiotics in the nutrition of ducks). *Bull. Acad. Vet. France* 29, 143-145.

It was concluded that antibiotics have no beneficial effect in the nutrition of ducks.

102. Luecke, R. W., McMillen, W. N. and Thorp, F., Jr., 1950, The effect of vitamin B₁₂, animal protein factor, and streptomycin on the growth of young pigs. *Arch. Biochem.* 26, 326-327.

A 10% increase in growth over that obtained with the basal ration was produced by adding 12.5 micrograms of B₁₂ per lb of feed. However, a 40% increase resulted when 0.5% streptomycin was added to the control ration. The APF supplement was as effective as the B₁₂ streptomycin preparation. No difference in the feed efficiencies of the various groups was observed.

103. Cunha, T. J., 1958, Antibiotics for swine, beef cattle, sheep and dairy cattle. First International Conference on Use of Antibiotics in Agriculture. National Research Council and National Science Foundation.

104. Braude, R., Wallace, H. D. and Cunha, T. J., 1953, The value of antibiotics in the nutrition of swine. A review. *Antibiotics and Chemotherapy*, 3, 271-291.

A fine review recommended to all who are interested in the details of antibiotics in swine nutrition.

105. Catron, D. V., Maddock, H. M., Speer, V. C. and Vohs, R. L., 1951, Effect of different levels of aureomycin with and without vitamin B₁₂ on growing-fattening swine. *Antibiotics and Chemotherapy* 1, 31-40.

During the first week, scouring occurred in some of the pigs regardless of their ration. In pigs on the two lower levels of aureomycin the scouring subsided after the first week; in some of those on the higher levels, with or without vitamin B₁₂, loose movements continued for two weeks, but their scouring was not typical.

From these and previous studies, the authors attribute the action of antibiotics in swine nutrition to specific inhibition of bacterial organisms

that compete with the animal for the nutrients in the ration, irritate the intestinal tract or produce toxins harmful to the pig.

106. Clausen, H., 1955, The influence of antibiotics on carcass quality when pigs fed antibiotics. First International Conference on the Use of Antibiotics in Agriculture. National Research Council and National Science Foundation, Washington, D. C., pp. 19-32.

Restricted or *ad libitum* feeding at optimal amount of proteins, minerals and vitamins greatly influences the carcass quality, especially with regard to the ratio of lean meat and fat. Pigs cannot be forced to produce more meat than the level determined by heredity and, therefore, a surplus of feed will be converted into fat.

107. Lasley, J. F., Pribble, L. F. and Hogan, A. G., 1954, Value of antibiotics in swine rations, Univ. of Missouri Agric. Exper. Sta. Bull. 543.

All four of the antibiotics tested (Aureomycin, procaine penicillin, streptomycin, chloromycetin) gave an increase in the rate of economy of gains when added to a corn soybean meal ration for growing fattening pigs in dry lot.

108. Speer, V. C., Vohs, R. L., Catron, D. V., Maddock, H. M. and Culbertson, C. C., 1950, Effect of aureomycin and animal protein factor on healthy pigs. *Arch. Biochem.* **29**, 452-453.

The addition of 5 or 10 mg of aureomycin per pound of basal ration failed to increase the daily weight gains of healthy pigs or to improve their feed efficiency. The failure of aureomycin to improve gains or feed efficiency might be explained by the "disease level" theory. Healthy, previously well-fed pigs, managed under disease free conditions, may not respond to aureomycin feeding as would unthrifty pigs fed in unsanitary surroundings.

109. Wahlstrom, R. C. and Johnson, B. C., 1951, Growth effect of various antibiotics on baby pigs fed synthetic rations. *Federation Proc.* **10**, 397.

The addition of aureomycin or of chloramphenicol at 100 mg/kg of dry matter consumed caused a significant increase in average daily gain over the basal group and also an increased cecal size. The addition of aureomycin to the diet of pigs not receiving vitamin B₁₂ did not prevent the symptoms of vitamin B₁₂ deficiency. Injection of crystalline vitamin B₁₂ to these pigs after 49 days on a deficient diet caused a significant reticulocytosis as well as an increase in average daily gain and feed efficiency.

110. Becker, D. E., Terrill, S. W., Ullrey, D. E. and Meade, R. J., 1952, The growth response of the pig to a dietary source of various antibacterial agents and to intramuscular injections of procaine penicillin. *Antibiotics and Chemotherapy* **2**, 259-264.

Aureomycin hydrochloride and procaine penicillin were of equal activity and stimulated a statistically significant increase in rate of gain over the control group. Neomycin and chloramphenicol produced only a slight increase in growth rate.

The intramuscular injection of an aqueous suspension of procaine penicillin G at the rate of 200,000 units on alternate days failed to influence the rate of gain or feed efficiency of pigs fed a practical diet containing animal by-products. However, the injection of a similar quantity of procaine penicillin G suspended in sesame oil containing 2%

(W. V.) aluminum monosterate gave a statistically significant (odds 18:1) increment in rate of gain.

111. Wahlstrom, R. C., Cohen, E. M. and Terrill, S. W., 1952, Growth effect of various antibiotics on baby pigs fed synthetic rations. *J. Animal Sci.* **11**, 449-454.

The antibiotic supplemented groups showed a three-fold increase in the cecal size.

In a second trial three groups of 3 pigs each were fed for 56 days a complete "synthetic milk" diet, alone or supplemented with either 100 mg of chloromycetin or 100 mg of aureomycin per kg of dry matter consumed. Average daily gains (lb) were as follows: Control diet 0.76; + aureomycin 0.83; + chloromycetin 0.86.

During the first nine days there was a marked decrease in coliform organisms in the feces of the pigs receiving chloromycetin; however, by the sixteenth day no difference was apparent. Chloromycetin also appeared to decrease lactic counts, although this decrease was not as marked as in the case of the coliform.

112. Horvath, D. J. and Vandernoot, G. W., 1954, Effect of three levels of a new antibiotic, tetracycline, in a swine ration. *J. Animal Sci.* **13**, 899-903.

Tetracycline at all levels used improved feed efficiency but did not improve rate of gains as compared to chlortetracycline and to the negative control. The increase in feed efficiency was greatest in the initial part of the trial.

113. Oldfield, J. E. and Hale, O. M., 1952, A comparison of methods of administration of penicillin to suckling pigs. *J. Animal Sci.* **11**, 772.

Weight gained from birth to weaning averaged 19.8 pounds among the controls; 23.3 pounds for the injected group; 21.5 pounds for those receiving one large dose orally and 20.2 pounds in the group which received the same amount of penicillin divided among four weekly oral doses. The results obtained indicate some advantage for antibiotic supplementation for pigs which are still nursing the sow; moreover they suggest the efficiency of parenteral administration.

114. Wallace, H. D., Albert, L. T., Ney, W. A., Combs, G. E. and Cunha, T. J., 1953, Effects of reducing and discontinuing aureomycin supplementation during the growing fattening period of pigs fed corn-peanut meal, corn soybean, meal, and corn-cottonseed meal rations. *J. Animal Sci.* **12**, 316-321.

Evidence is provided indicating that antibiotic supplementation should not be discontinued during the growing fattening period of the pigs if optimum gains are to be obtained.

115. Burnside, J. E., Grummer, R. H., Phillips, P. H. and Bohstedt, G., 1953. The effect of intermittent administration of Aureomycin to growing-fattening swine. *J. Animal Sci.* **12**, 828-835.

Deletion of the antibiotic from the ration during the middle or latter part of the feeding caused concomitant decrease in rate of gain.

116. Clauson, A. J., Sheffy, B. E. and Willman, J. P., 1953. The value of implanted antibiotic pellets for suckling pigs. *J. Animal Sci.* **12**, 911.

In this study, the various antibiotics as implanted had no influence on average daily gain of nursing pigs.

117. Swenson, M. J., Buckner, R. G., Goetsch, D. D., Aubel, C. E. and Underbjerg, G. K. L., 1954, Growth of newborn pigs implanted subcutaneously with bacitracin pellets. *J. Animal Sci.* **13**, 1032.

Growth of the nursing pigs was not altered by the implanted antibiotic pellets.

118. Terrill, S. W., Becker, D. E., Grad, D. I. and Lassiter, J. W., 1953, Effect of subcutaneous implantation of antibiotic pellets on the growth and survival of suckling pigs. *Antibiotics and Chemother.* **3**, 1011-1014.

Under the conditions of the tests, the subcutaneous implantation of bacitracin, penicillin or chlortetracycline pellets did not significantly alter either weaning weights or survival of pigs to weaning age. The presence of an antibiotic in the pig starter ration did not affect the survival weaning weights or the response to procaine penicillin G (25 mg) implants.

119. Perry, T. W., Thrasher, G. W. and Beeson, W. M., 1953, The effect of bacitracin implants on weaning weights and subsequent feed lot performance of two-day old pigs. *J. Animal Sci.* **12**, 824-827.

The subcutaneous implantation of a pellet containing 22.5 mg bacitracin in 2-day-old crossline pigs had no effect on 42 day weaning weights or subsequent feed lot performance whether or not the post-weaning ration contained 7.5 mg aureomycin hydrochloride per pound total ration.

120. Wallace, H. D., McKigney, J. and Gillespie, L., 1954, The influence of subcutaneous implantation of bacitracin and chlortetracycline (aureomycin) pellets on the growth and survival of suckling pigs. *Antibiotics and Chemother.* **4**, 663-665.

Two hundred and thirty pigs from 28 litters were used in two separate experiments to study the influence of bacitracin (1,000 units) and chlortetracycline HCl (20 mg) pellets implants on growth and survival of nursing pigs. In these tests, there was no significant effect of either antibiotic on four-week weights, 56-day weights, or survival from birth to weaning.

121. Lasley, J. F., Tribble, L. F., Case, A. A. and Penrod, E. J., 1953, The influence of porcine gamma globulins and antibiotic pellets on the growth and survival of baby pigs. *J. Animal Sci.* **12**, 923.

In the first experiment involving 142 pigs, those receiving bacitracin pellets at 2 days of age average 33.66 lb at 56 days as compared to 31.50 lb for litter mate controls. This difference was significant ($P = 0.01$) but there was no significant difference in mortality between the two groups of pigs. When bacitracin pellets were not implanted until the pigs were 2 weeks of age, they had no effect on weaning weights of the pigs.

122. Becker, D. E. and Terrill, S. W., 1954, Various carbohydrates in a semi-purified diet for the growing pig. *Arch. Biochem. and Biophys.* **50**, 399-403.

The different carbohydrates constituted 50% of the diet. Glucose, sucrose, dextrin and corn starch produced equally satisfactory results. However, lactose-fed pigs exhibited a depressed feed intake, slow growth and a moderate diarrhea, but the efficiency of gains was equal to that of

pigs fed other carbohydrates. At 16 weeks of age, the pig was able to tolerate 25% lactose in the diet without harmful effect.

123. Loosli, J. K., Wasserman, R. H. and Gall, L. S., 1951, Antibiotic studies with dairy calves. *J. Dairy Sci.* **34**, 500.

For 39 pairs of calves studied, the average daily gain in weight during the 56-day period was 1.16 lb for those fed the antibiotic supplement and 0.95 lb. for the control calves. The difference in rate of gain was statistically significant (odds 99:1). There were no significant differences in the amounts of hay consumed, but the calves fed the antibiotic consumed approximately 40% more concentrate than the controls and they required less T. D. N. to make 1 lb of gain. Control calves exhibited a greater incidence and severity of scours than those fed the antibiotic.

124. Murley, W. R., Jacobson, N. L., Wing, M. J. and Stoddard, G. E., 1951, The response to aureomycin supplementation of young dairy calves fed various "practical" and restricted diets. *J. Dairy Sci.* **34**, 500.

In each group the aureomycin-fed calves were superior in weight gains and in physical appearance to the controls. Among the calves in group I scouring was infrequent, and since the calculated TDN intake of these calves were similar, an increased efficiency of feed utilization (TDN intake/lb weight gain) due to aureomycin feeding is indicated.

125. Rusoff, L. L. and Davis, A. V., 1951, Effect of aureomycin on growth of young calves weaned from milk at an early age. *J. Dairy Sci.* **34**, 500.

The results for the first 90 days of this trial definitely indicate that aureomycin produces a growth stimulation in young calves. The Jersey calves receiving the aureomycin feeding supplement and those receiving the crystalline aureomycin showed a gain of approximately 25% over the Jersey control calves; the Holstein aureomycin groups showed a gain of approximately 15% over the Holstein control group. The antibiotic supplementation appeared to lower the incidence of scours.

126. Porter, J. W. G., 1953, Antibiotics and nutrition. *Vet. Rec.* **65**, 288-290.

Penicillin has not been extensively tested in the United States (in cattle) but experiments now in progress at Shinfield indicate that satisfactory responses are obtained with either penicillin or Aureomycin.

127. Clauson, G. R., Musgrave, S. D., Norton, C. L. and Gallup, W. D., 1953, alfalfa vs. prairie hay for calves with and without aureomycin supplement. *J. Dairy Sci.* **36**, 594 (in Soc. Proc.).

Aureomycin improved the growth rate of calves.

128. Hibbs, J. W. and Conrad, H. R., 1953, The effect of feeding aureomycin supplement on the performance of calves raised on the high roughage system. *J. Dairy Sci.* **36**, 593-594.

Aureomycin feeding resulted in greater weight gains, higher feed intake and more efficient utilization of feed measured by TDN/lb of gain. From 8 to 12 weeks the Aureomycin-fed calves maintained an average blood sugar level of approximately 9 mg/100 higher than the controls. While no difference in the average total steam volatile fatty acids or acetic acid in the rumen juice was found at 12 weeks of age propionic acid was slightly lower and butyric acid slightly higher in the aureomycin fed group.

129. Rusoff, L. L., Fussell, J. M., Hyde, C. E. and Crown, R. M., 1953, Oral supplementation versus intramuscular injection of aureomycin to young calves. *Dairy Sci.* **36**, 593.

Both aureomycin groups of calves significantly outgained the control group.

130. Rusoff, L. L., Fussell, J. M., Hyde, C. E., Crown, R. M. and Gall, L. S., 1954, Parenteral administration of Aureomycin to young calves with a note on mode of action. *J. Dairy Sci.* **37**, 488-497.

Parenteral administration (intramuscular injection) of 400 mg of Aureomycin to young calves once weekly increased growth by 30% over controls at 16 weeks of age. Aureomycin administered orally at a level of 50 to 90 mg daily stimulated growth by 20%. The antibiotic also caused a significant increase in height at withers.

131. Richardson, C. W., Ronning, M., Berousek, E. R. and Norton, C. L., 1953, The effect of Aureomycin upon the growth of dairy calves when administered orally, subcutaneously, and intramuscularly. *J. Dairy Sci.* **36**, 593.

These results indicated that oral administration at both levels was effective in increasing body weight gain as compared to controls at the same age. Parenteral administration of Aureomycin at the rate of 60/mg/week did not affect the growth rate under these conditions.

132. Lassiter, C. A., 1955, Antibiotics as growth stimulants for dairy cattle: a review. *J. Dairy Sci.* **38**, 1102-1138.

The antibiotics aureomycin and terramycin are the only ones which have been studied sufficiently to warrant valid conclusions. Considerably more research needs to be conducted with Terramycin before conclusions concerning its use can be accepted with the same degree of confidence as recommendations for aureomycin.

133. Knodt, C. B. and Ross, E. B., 1953, Penicillin in milk replacements for dairy calves. *Proc. Soc. Exper. Biol. and Med.* **82**, 663-665.

Feeding of 0.5 gm potassium penicillin per 100 lb of milk replacement to Holstein bull calves resulted in a decreased rate of gain in body weight and growth in height at the withers in this experiment.

134. Neumann, A. L. and Snapp, R. R. and Gall, L. S., 1951, The long-time effect of feeding aureomycin to fattening beef cattle, with bacteriological data. *J. Animal Sci.* **10**, 1058-1059.

No benefit was obtained when aureomycin, either in crystalline form or as a crude concentrate containing residual vitamin B₁₂ (Aureofac), was added to a basal fattening ration of corn silage, ground yellow corn, linseed meal, and salt, fed to 18 yearling beef heifers for 150 days. Neither did the low level fed (2 mg aureomycin per pound air dry feed) bring about any extremely unfavorable physiological disturbances. Severe reduction in appetite was shown for a few days but partial recovery was made, and for the entire 150 days the heifers receiving aureomycin voluntarily consumed a daily ration equal to approximately 2% of their body weight.

135. Fincham, R. C. and Voekler, H. H., 1953, The longtime effects of aureomycin feeding to dairy heifers. *J. Dairy Sci.* **36**, 594.

Maximum growth stimulation from Aureomycin feeding was observed from birth to 6 months.

136. Jordan, R. M. and Bell, T. D., 1951, Effect of aureomycin on growing and fattening lambs. *J. Animal Sci.* **10**, 1051.

The effects of small amount of aureomycin (5 to 15 mg daily) on the rate of suckling and fattening lambs were studied. Ten suckling lambs were drenched with 5 mg of aureomycin daily for six weeks. During this time they gained an average of .65 lb daily as compared to ten control lambs that gained .54 lb daily. Four other lambs were drenched with 15 mg and made an average daily gain of .59 lb. All the lambs receiving aureomycin were normal in every respect. Two trials were conducted of fattening lambs fed a standard corn-alfalfa ration and supplemented with 6-12 mg of aureomycin per lamb daily. During the first preliminary trial, 21 lambs fed 6 mg of aureomycin gained .49 lb per lamb daily as compared to .39 lb per lamb daily for the controls. The treated lambs required 22% less concentrates per 100 lbs. of gains.

137. Evans, J. L., Grainger, R. B. and Thompson, C. M., 1957, The effect of different levels and prolonged supplementation of chlortetracycline upon roughage digestion by sheep. *J. Animal Sci.* **16**, 110-117.

Lambs (65 lb) fed 5 or 10 mg chlortetracycline per pound of feed showed loss of appetite and decreased crude fiber digestibility. No data on weights.

138. Andrews, E. D., Gall, L. S. and Hart, L. I., 1957, Effects of large oral doses of penicillin on pasture-fed lambs. *New Zealand J. Sc. Technol.* **38A**, 649-653. (*Chem. Abstr.* **51**, 13092, 1957.)

Four pasture-fed lambs averaging 40 lb body weight were each given 150,000 units of crystalline sodium penicillin G one day, a second like dose after an interval of three days and a third dose after a further interval of two days. Seven days after the first dose the treated lambs had on the average, lost weight, whereas the two controls had an average gain. Treated lambs showed decreased appetite and increased thirst. Examination of rumen specimens showed no definite evidence that penicillin had affected rumen vitamin B₁₂ activity: however, in the specimens from treated animals EH values were depressed (decreased anaerobiosis), fiber digesting ability was impaired, and Gram stains became atypical. Within 14 days after withdrawal of the penicillin an apparent complete recovery (as evaluated by weight gains and rumen-function tests) occurred.

139. Gordon, W., 1956, First International Conference on Antibiotics in Agriculture. National Academy of Sciences and National Research Council, Washington, pp. 153-160.

Aureomycin increased the growth of foals through one year. Growth response was seen in pigs whether the antibiotic was given by mouth or by injection. He proposes one mode of action of antibiotics is in simple prophylaxis against infection, clinical and subclinical.

140. Taylor, J. H., Gordon, W. S. and Burrell, P., 1954, The effect of supplementing the diet of thoroughbred foals with Aureomycin hydrochloride. *Vet. Rec.* **66**, 744-748.

A supplement of 50 to 100 mg of Aureomycin hydrochloride was administered twice daily to a controlled group of 6 thoroughbred foals for the first nine months of life. During the period of supplementation a significant increase in growth rate occurred.

There was no improvement in the utilization of food as measured by the intake of corn. There was no alteration in bone development discernible by x-ray examination.

141. Clifford, R. J., Henderson, G. N. and Wilkins, J. H., 1956, The effect of feeding penicillin and vitamin B₁₂ to mature debilitated horses. *Vet. Rec.* **68**, 48-51.

When 6 adult horses, all showing some degree of poor physical condition, were given a feed supplement containing 1,025,000 units of penicillin in 1 lb of distillers spent yeast, no visible effect was seen, as compared to 6 additional horses in the same condition fed normal ration without penicillin supplement.

142. Berthelon, M., 1953, Facteurs de croissance chez le poulain (Growth factors for foals). *Rev. Med. Vet. Lyon et Toulouse* **16**, 1-4.

Vitamin B₁₂, Aureomycin and penicillin did not increase the rate of growth of foals 6 to 8 months of age when fed in amounts which increased the growth rate of pigs (about 3 to 5 mg/kg).

143. Schonherr, W., 1956, Zum nutritiven Wirkungsmechanismus von Antibiotica bei Tieren (Nutritive mechanism of the action of antibiotics in animals). *Naturwissen schaften* **43**, 330-331. (*Nutrition Abstr. and Rev.* **27**, 2363, April 1957.)

Puppies fed on an adequate mixed animal and vegetable diet and given 25 mg aureomycin as 3.125 gm Aurofac 2A per animal daily from 4 to 12 weeks of age weighed on the average 1275 gm, 18% more than littermates not given aureomycin. The blood of dogs which had different doses of aureomycin or terramycin for different periods did not differ significantly from that of controls in Hb value, sp. gr., osmotic resistance, sedimentation rate, or erythrocyte, leucocyte, thrombocyte and differential leucocyte count.

144. Klussendorf, R. C., Bacitracin and penicillin in the feed. *Southwestern Vet.* **9**, 124-127. Winter.

High level feeding of antibiotics to poultry and turkeys has become an accepted practice.

Antibiotic feeding to calves gives good results during the first eight weeks, but the advantage gained during the early weeks often is no longer present when the calves have reached an age of 4 to 6 months.

In puppies, the addition of antibiotics to the feed has proved disappointing, weight increases are due primarily to fat, while skeletal and muscular tissues do not gain from adding antibiotic to the ration.

145. Arnrich, L., Lewis, E. M. and Morgan, A. F., 1952, Growth of dogs on purified diet plus aureomycin and/or vitamin B₁₂. *Proc. Soc. Expt. Biol. and Med.* **80**, 401-404.

One of two pups fed 100 ppm chlortetracycline grew better than control pups.

146. Watt, R. P., 1953, Mink nutrition research, Oregon Agric. Exper. Sta. Prog. Rept. 3.

In breeding and reproduction tests, adding two levels of aureomycin to the feed of mink had little or no effect on breeding. A significantly lower kit-per-litter average, however, was seen in the antibiotic-fed groups.

In growth experiments it was found "that mink respond later in their

growth period to terramycin than to aureomycin." On the basis of these experiments, however, it has been found that overall responses of mink was greater to terramycin than to aureomycin. The response to Aureomycin was greater when fed from the period when the kits began to eat solid food through pelting as compared to when fed from July 1 through pelting.

147. Bassett, C. F., Travis, H. F., Warner, R. G. and Loosli, J. K., 1957, Antibiotics in feeds increase mink pelt size. Cornell Feed Service No. 50, pp. 7-8.

Experiments over a two-year period have shown conclusively that the addition of 3 mg of either aureomycin or terramycin to the ration (as fed basis) of growing mink kits will result in larger mink and larger pelts.

148. Norofeldt, S., Melin, G. and Thelander, B., 1954, Forsök med antibiotika i fodret till palsdjur (Experiments with antibiotics in the ration of fur animals). Kungl. Lantbrukshögsk. Statens Husdjursforsök Sartryck. No. 107, 24 pp. (*Nutrition Abstr. and Rev.* 26, 1269.)

The effects of aureomycin, terramycin, bacitracin, penicillin and vitamin B₁₂ on pregnant and nursing mothers of silver fox, blue fox and mink and on their kits up to pelting times were observed. Results varied considerably. Weaned silver fox and blue fox kits fed on an all-vegetable diet did not grow normally and the addition of antibiotics or vitamin B₁₂ had no effect. Fresh meat or fish was needed for good growth and antibiotics gave little improvement but were beneficial to suckling young. Procaine penicillin prevented diarrhea and weight loss in mink kits fed for several days on tainted fish.

149. Breirem, K., Hoidsten, H. and Hoie, J., 1955, Antibiotics in animal nutrition. Report to the European Symposium on Antibiotics and New Growth Factors in Animal Nutrition, Rome, May 1955.

Antibiotics gave no consistent growth advantage during experiments which extended over a two-year period with both mink and fox.

150. Enterocolitis in chinchillas fed chlortetracycline. *NCBA Res. Bull.* 27, Sept. 1956. (Abstr. *J. A. V. M. A.* 130, 102, 1957.) Anon.

Fifty of 450 chinchillas fed pellets containing 0.2 mg of chlortetracycline per ounce for 12 to 18 months died with a characteristic symptom complex. They became lethargic, refused feed and water, developed a diarrhea occasionally streaked with blood, and died quietly within 12 to 36 hours.

Necropsy revealed liquid fecal material but no gross lesions. Multiple areas of focal necrosis and shallow ulcers covered by pseudomembrane were seen microscopically in the large intestine and occasionally in the small intestine. They contained myriads of gram-positive cocci in huge clumps, but no parasites nor fungi, and often no gram-negative bacteria.

All antemortem stool and throat cultures showed profuse growth of hemolytic *Staphylococcus aureus* but no *Shigella* or *Salmonella*.

The condition was corrected by changing to pellets which contained no antibiotics and by giving for four days, neomycin (50 units/ml) and bacitracin (25 units/ml) in the drinking water; neither is appreciably absorbed and both destroy *Staphylococcus*.

151. Lawrence, J. M. and McGinnis, J., 1952, The effect of terramycin on the growth of rabbits. *Arch. Biochem. and Biophysics*, **37**, 164-166.

The addition of 1 to 50 ppm terramycin to the pelleted feed for weanling rabbits failed to increase the growth rate during a six-week experimental period. An all plant protein ration supplemented with B₁₂ was used in these trials.

152. Huang, T. C., Ulrich, H. E. and McCay, C. M., 1954, Antibiotics, growth, food utilization and use of chromic oxide in studies with rabbits. *Federation Proc.* **13**, 462.

In antibiotic studies, contrary to ordinary belief, supplementation with either commercial or synthetic forms of aureomycin and terramycin to a pelleted natural ration gave no growth stimulus to young growing rabbits. Rations either low in protein or an essential vitamin, niacin, were not improved by supplementation with terramycin.

153. Huang, T. C., Ulrich, H. E. and McCay, C. M., 1954, Antibiotics, growth, food utilization, and the use of chromic oxide in studies with rabbits. *J. Nutrition* **54**, 621-630.

When terramycin or aureomycin was fed at levels ordinarily recommended for other species of animals, no growth improvement was shown for young rabbits fed a pelleted natural ration. Terramycin did not improve growth when the diet contained 0.5% sulfathalidine. This antibiotic also was ineffective in improving the growth of rabbits which received a semipurified ration containing either 25% casein or 24.7% soy protein, plus 0.5% methionine.

154. Hewes, C. G., 1955, The influence on the growth and progeny of the guinea pigs resulting from oral administration of aureomycin (chlortetracycline) and penicillin. *J. Nutrition* **57**, 353-360.

First-litter progeny of animals treated with antibiotics showed a slight increase in mean birth weight over that of first-litter progeny of control animals. However, this increase was not apparent after six weeks of administration of the low level of 0.1 mg of the drugs to the young. No other effects on reproduction were observed. Male guinea pigs fed 0.3 and 0.5 mg of aureomycin per day showed a significant average weight gain over the controls after a period of nine weeks. Of the organs and tissues removed for study, only the tibias of the experimental animals weighed significantly more than those of the controls. The heart and spleen weighed significantly less in the antibiotic-fed animals than in the controls. Sections of these organs and tissues indicated no apparent histological differences between experimental and control animals. Increased structural growth was noted in the great length of the tibia in the aureomycin-fed animals than in the controls.

155. Oleson, J. J., Hutchings, B. L. and Whitehill, A. R., 1950, The effect of feeding aureomycin on the vitamin B₁₂ requirement of the chick. *Arch. Biochem.* **29**, 334-338.

The interdependence of the growth effects of vitamin B₁₂ and aureomycin in the chick were studied. Each factor appears to have a sparing action on the other.

Preliminary experiments with rats are described which show that several antibiotics (aureomycin, penicillin, streptomycin, and chloramphenicol) are capable of stimulating the growth of this species. The

growth-stimulating effect of the antibiotics appears to be an indirect mechanism and not dependent upon the structure of the antibiotics.

156. Mirone, L., 1953, Effect of aureomycin and terramycin on growth, reproduction and blood count in mice. *Antibiotics and Chemother.* 3, 600-602.

The addition of aureomycin HCl to a purified diet containing 23% casein, improved the growth of weanling mice. At the levels used, Aureomycin HCl (25, 50 and 100 mg/kg feed) did not improve reproduction performance and had no deleterious effects on the blood count.

157. Vijayaroghaven, P. K., Murphy, E. A. and Dunn, M. S., 1952, The effect of aureomycin on the growth of mice. *Arch. Biochem. and Biophys.* 36, 127-31.

Mouse growth stimulated by antibiotics when fed soybean meal or cottonseed meal diets, not when fed casein or peanut meal diets.

158. Dickinson, C. D. and Scott, P. O., 1954, The effects of adding penicillin and aureomycin to the diet of cats. *Brit. J. Nutrition* 8, 380-385.

The addition of penicillin and aureomycin to a diet containing 50% protein, mostly of animal origin, produced increased growth in kitten, accompanied by increased food intake and increased efficiency of food conversion, greater freedom from infection and an improvement in general health.

159. Schumacher, R. E., 1955, Growth of brown trout (*Salmo trutta fario*) fingerlings on a diet fortified with aureomycin and thiamin hydrochloride. *Progressive Fish Culturist* 17, 123-125. (*Chem. Abstr.* 49, 16241, Nov. 25, 1955.)

When fed 3 to 5 mg aureomycin daily per pound of trout and 4.5 to 8.0 mg of thiamine-HCl for 16 weeks, together with an adequate diet of 45% beef spleen and 55% dry cereal mixture, trout showed no significant growth or weight increases over controls.

160. Wagner, E. D., 1954, Effects of antibiotics and arsenilic acid on the growth of rainbow trout fingerlings. *Progressive Fish Culturist* 16, 36-38. (*Chem. Abstr.* 48, 12324-12325.)

Growth increases in young *Slamo gairdneri* as a result of feeding terramycin, chloromycetin, aureomycin, penicillin and arsanilic acid were negative.

161. Murphy, M. R. V. and Screenivasaya, M., 1953, Effect of antibiotic in the growth of the silkworm, *Bombyx mori* L. *Nature* 172, 684.

162. Sharada, K. and Bhat, J. V., 1956, Effect of chloromycetin and glycine on the growth and production of silk by *Bombyx mori* L. *Jour. Indian Inst. Sci.* 38, 136-147. (*Biol. Abstr.* 31, 2824, Jan. 1957.)

Two batches of silkworms, one in the 4th instar and the other in the 5th, were fed on alternate days with various concentrations of chloromycetin and the minimum quantity of the antibiotic required to produce the maximum beneficial effect was 50-60 mg/kg body weight of the larvae. Supplementing chloromycetin with glycine possessed the distinct advantage of reducing the quantities of the 2 substances to half the concentrations previously employed to produce the desired effects. It was also indicated that administration of chloromycetin to the larvae twice on alternate days is less effective than supplementing the antibiotic once daily with glycine.

163. Palmer, A. Z., Chapman, H. L., Jr., Carpenter, J. W. and Alsmeyer, R. H., 1957, Slaughter, carcass and tenderness characteristics as influenced by feed intake of steers fed chlortetracycline and/or diethylstilbestrol on pasture and in dry lot. *J. Animal Sci.* **16**, 1075.

Steers on each of four feeding programs were subdivided into four lots of 8 steers each. One lot received 90 mg of aureomycin per steer daily in the ration, one lot received 10 mg of stilbestrol, one lot received stilbestrol plus aureomycin while the control lot received no additives. Percentages of hide and liver yield cooler shrinkage, carcass grade, marbling, carcass length, thickness of chuck, length of leg, circumference of round and ether extract of the longissimus dorsi muscle at the 13th rib and tenderness by shear and panel were obtained. Breed differences were significant for all traits except area of longissimus dorsi and tenderness. Breed differences in panel tenderness score approached significance. The feeding of aureomycin or stilbestrol showed no significant effect on any trait.

164. Rusoff, L. L., Landagora, F. T. and Hester, H. H., 1954, Effect of aureomycin on certain blood constituents (Hb, packed with RBC, Ca, P, RBC and WBC), body temperatures, weights of organs and tissues, and thickness of small intestine. *J. Dairy Sci.* **37**, 654.

Preliminary results indicate: a lowered body temperature in Holstein calves receiving aureomycin; no apparent differences between the groups of animals in erythrocyte counts, per cent hemoglobin, per cent hematocrit value, plasma Ca and plasma inorganic P values; and an indication of a decrease in leukocyte counts for the aureomycin-supplemented calves. Certain organs (pituitary thyroid, thymus, liver, etc.) were heavier in the aureomycin calves; however, no differences were observed in organ weights when computed as percentage of body weight. A decrease in thickness of duodenal and jejunal sections of the intestinal wall and an increase in the ileal section were found in the aureomycin-supplemented calves.

165. Trams, E. G., Kasiwa, H. K., Cornman, I. and Klopp, C. T., 1955, Effects of large doses of parenterally injected chlortetracycline on the adrenal glands of rats. *Antibiotic Medicine* **1**, 677-688.

Chlorotetracycline displayed ACTH-like activity in rats. It stimulated adrenal cortex activity, decreased adrenal cholesterol, reduced circulating eosinophils, raised free gonadal hormones in urine and increased adrenal and liver weight.

166. Gordon, H. A., 1952, A morphological and biochemical approach. A Colloquium. Studies on the Growth Effect of Antibiotics in Germfree Animals. Notre Dame, University of Notre Dame, Indiana.

Conventional chicks fed antibiotics appeared to have a thymic stimulation and an intestinal depression, "in the conventionals under the effect of antibiotics drugs, the intestinal wall is practically stripped down to the tissues necessary for absorption. There seems to be little interference of cellular elements necessary for defense and other purposes."

167. Burgess, R. G., Gluck, M., Brisson, G. and Laughland, D. H., 1951, Effect of dietary penicillin on liver vitamin A and serum carotenoids in the chick. *Arch. Biochem.* **33**, 339-340.

Sodium Penicillin G (30 mg/kg) increased the vitamin A content of both serum and liver.

168. Burgess, R. C., Gluck, M. and Laughland, D. H., 1953, Biochemical changes in the penicillin-fed chick. *Poultry Sci.* **32**, 444-449.

Addition of penicillin (3 mg/kg) to the diet had an inconsistent effect on growth.

Although liver weight per kg body weight was less and dry matter greater in birds fed the penicillin-supplemented ration for 30 days, there was no significant difference in the protein ether-extractable or cholesterol fractions of the liver of control and penicillin-fed chicks on either a percentage dry liver or per kg body weight basis. In this group penicillin also significantly increased the liver vitamin A concentration per gram dry liver and per kg body weight. No significant change in the concentration of any of these constituents was observed in birds on a test of 15 days where penicillin also failed to enhance growth.

The antibiotic did not affect packed cell volume, hemoglobin, plasma, non-protein nitrogen, creatinine, glucose, total fatty acid or cholesterol levels. It did elevate plasma carotenoid and ascorbic acid levels and caused a small but highly significant rise in plasma-phospholipid phosphorus concentration which was independent of a growth response to the antibiotics.

169. Braude, R., Coates, M. E., Davies, M. K., Harrison, G. F. and Mitchell, K. G., 1955, The effect of aureomycin on the gut of the pig. *British J. Nutrition* **9**, 363-368.

Aureomycin was given in a normal fattening ration at the rate of 20 gm/ton from weaning until slaughter at pork or bacon weight.

Immediately after slaughter the weights and lengths of the small intestines were measured. A few of the intestines were examined for histological changes. Determinations of fat, moisture and total nitrogen were made on the gut of nine pairs of pigs, and the liver and kidneys and spleen of these animals were also weighed.

In all experiments there was a tendency for gut weight, adjusted to constant body weight, to be reduced in the aureomycin-supplemented pigs.

There was no significant effect on the weights of the liver, kidneys or spleen, nor was there any consistent effect on gut length, which indicated that the reduction in weight was due to a thinning rather than to a shortening of the gut. No chemical or histological changes in the intestines were detected.

170. Dickson, W. M., Patterson, E. B., Stern, J. R. and McGinnis, J., 1954, The effect of terramycin or fish solubles, or both, on the growth, adrenal glands and gonads of the rat. *J. Nutrition* **54**, 631-641.

Terramycin failed to stimulate growth of the normal healthy rat to a significant degree. In an experiment in which surgery (castration or sham-castration) was performed 14 to 20 days following weaning, significant ($P = 0.05$) growth stimulation was obtained after 49 days on the experimental diet. It is suggested that terramycin acted as a growth stimulant only to the animal subjected to stress. There was some indication that both terramycin and fish solubles caused an increase in the weight of the adrenal gland in the growing rat,

Neither supplement affected the weight of the male sex organs of the growing rat.

171. Gordon, H. A., Wagner, M. and Westmann, B., 1957, Studies on conventional and germfree chickens treated orally with antibiotics. *Antibiotic Ann.* 248-255.

Confirmation of the previous work of this group and others on the decreased gut weight noted in chicks fed antibiotics. Germfree chicks fed 50 mg procaine penicillin G did not give a positive response.

172. Menge, H. and Conner, M. G., 1955, Effect of chlortetracycline on chick thyroid size. *Proc. Soc. Exptl. Biol. and Med.* 88, 216-218.

Chlortetracycline was fed to chicks at levels of 10, 50, 500 and 1,000 mg/kg of diet. The data show that this treatment significantly increased the size of the thyroid gland. Antibiotic dosage up to 100 mg/kg of diet was correlated to increase the thyroid size. Increasing the antibiotic dosage above this level did not increase the thyroid size at the same rate. Since the growth of the chicks was not depressed and the thyroid was not enlarged as much as it was when thiouracil was fed, it appears that chlortetracycline did not duplicate the action of thiouracil.

173. Calesnick, B., Harris, W. D. and Jones, R. S., 1954, Antithyroid action of antibiotics. *Science* 120, 128-229.

Feeding antibiotics increased the weight of the thyroid glands.

174. Mellen, W. J. and Waller, E. F., 1954, Antibiotic and thyroid size in growing chickens. *Poultry Sci.* 33, 1036-1037.

Four groups of chicks were fed for eight weeks a standard broiler ration supplemented as follows: 1. none; 2. chlortetracycline 75 gm/ton feed; 3. bacitracin 100 gm/ton feed; and 4. furazolidone 100 gm/ton feed.

Thyroids in the three treated groups were considerably larger than those in the controls. Treated birds also weighed more than the controls.

175. Grant, W. C., 1954, Aureomycin and the thyroid gland. *Science* 120, 724-725.

The addition of 1 mg of chlortetracycline per kg of a mixed animal-plant protein diet did not produce any significant changes in the weight of the thyroid gland of rats after 42 days of feeding.

176. Lee, C. C., Harris, P. W., Anderson, R. C. and Chen, K. K., 1957, Absence of thyroid changes by erythromycin in chickens. *Antibiot. and Chemother.* 7, 132-134.

Erythromycin stimulated the growth of chicks but had no effect on the weight of histologic appearance of the thyroid gland.

177. Libby, D. A. and Meites, J., 1954, Negative effect of antibiotics on thyroid gland. *Science* 120, 354.

Thirty rats divided into three groups of 10 each were fed either a basal diet alone, the basal plus 50 mg/kg potassium penicillin G or the basal plus 50 mg/kg aureomycin. Neither of these antibiotics fed for 21 days altered the size of the thyroid gland, although aureomycin as well as penicillin increased the body weight of the animals.

In a second experiment, White Leghorn cockerels were fed either a basal ration or the same diet supplemented with 2 gm penicillin per ton of feed. By the end of the five week experiment period, penicillin had

failed to alter the thyroid weight of the chicks and in this experiment, it did not affect the body weight either.

178. Barber, R. S., Braude, R., Mitchell, K. G., Roak, J. A. F. and Rowell J. G., 1957, Further studies on antibiotics and copper supplements for fattening pigs. *Brit. J. Nutr.* **11**, 70-79.

Copper sulfate or chlortetracycline either had a tendency to reduce gut weight and length in pigs. Chlortetracycline did reduce the spleen weight.

179. Coates, M. E., Davies, M. K. and Kon, S. K., 1955, The effect of antibiotics on the intestine of the chick. *British J. Nutrition* **9**, 110-119.

Procaine penicillin in mash increased the growth rate of chicks, maintained in brooders, but reduced the weight and to a lesser extent the length of the small intestine. No histological changes were noted to account for the lowered gut weight. Moisture and fat content of the gut were not significantly altered by the penicillin treatment.

The authors state that the physiological effects of antibiotics in chicks reared on "infected" premises might be brought about by an alteration in the metabolic activity rather than the numbers of organisms in the gut. They believe that these data and that of other investigators lend support to the view that thickening of the gut is part of a defense mechanism, for if the absorption of harmful toxins is reduced the absorption of essential nutrients may of necessity be impaired as well.

180. Kimbrel, K. H., Fischer, W., Bunte, H. and Frik, W., Untersuchungen über enterale Antibiose bei der Ratte. 8. Der Einfluss von Tetracyclingenaben auf die Magen-Darm-Passage (Studies on intestinal antibiotics in the rat. 8. Effect of tetracycline on the rate of transit through the digestive tract). *Ztschr. ges. exper. Med.* **126**, 587-595, 1955-56. (*Nutrition Abstr. and Rev.* **26**, 3127, July 1956.)

After a single dose of aureomycin, the rate of passage of the barium meal was increased, especially in the upper part of the digestive tract, and after 14 days the rate of passage was almost normal and after 26 days it was significantly slower than normal, especially the emptying of the stomach. Again aureomycin had more effect than terramycin. Three days of treatment with vitamin B₁₂ restored the rate of passage to normal.

Isolated loops of small intestine from rats showed a fall in tonus and increased activity in the presence of aureomycin solution.

181. Jukes, H. G., Hill, D. C. and Branion, H. D., 1956, Effect of feeding antibiotics on the intestinal tract of the chick. *Poultry Sci.* **35**, 716-823.

Microscopic examination of serial section of the duodenum of antibiotic fed birds and of controls revealed that sections from birds receiving antibiotics had on the average a smaller diameter, a thinner tunica propria layer and shorter villi. The observed differences in the diameter and the thickness of the tunica propria layer were statistically significant. A measurement of the combined muscularis, muscularis mucosae, tunica propria and villus was less for the sections from birds receiving antibiotics.

182. Anderson, G. W., Cunningham, J. D. and Slinger, S. J., 1952, Effect of protein level and penicillin on growth and intestinal flora of chickens. *J. Nutr.* **47**, 175-198.

The addition of penicillin to the diet resulted in a considerable re-

duction in pH of the cecal contents. In the control groups pH ranged from 6.32–6.25, whereas in the penicillin-fed lots the values ranged from 5.90–5.62.

183. Stern, J. R., Gutierrez, J. C. and McGinnis, J., 1952, Detergency challenges antibiotics to explain penicillin growth stimulus. *Chem. and Engineering News* 30, 1374.

The intestinal contents of penicillin-fed chicks had significantly lower surface tension than those of the controls. They concluded that the action of antibiotics which stimulates growth also lowers surface tension.

184. Perdue, H. S., Spruth, H. C. and Frost, D. V., 1957, Effect of growth promotants on growth and intestinal weight of rats. *Federation Proc.* 16, 393.

Penicillin V, 0.01%; erythromycin sulfamate, 0.01%; synematin B, 0.01%; K-pen-G, 0.01%; and penicillin S, 0.0075%, significantly increased growth and lowered intestinal weight. Copper sulfate, 0.01%; DL-penicillamine, 0.008%; and copper arsenilate, 0.026%, decreased intestinal weight but did not increase growth. This indicates that decreased intestinal weight does not necessarily lead to increased growth. Contrary to findings with other species, copper sulfate, penicillamine and inactivated penicillin did not promote growth of rats.

185. McCoy, E., 1954, Changes in the host flora induced by chemotherapeutic agents. *Ann. Rev. Microbiol.* 8, 257–272.

186. Combs, G. F., 1956, Mode of action of antibiotics in poultry. First International Conference on Antibiotics in Agriculture. National Academy of Sciences and National Research Council. Washington, pp. 107–125.

Observations on changes in types and numbers of bacteria in the intestinal tract of chicks are inconclusive.

187. Johansson, K. R., 1955, Mode of action of antibiotics on animal growth. First International Conference on Antibiotics in Agriculture, *ibid.*, 127–134.

The classical microbiological methods, e.g., plate counts, pure culture studies, etc., are inadequate to obtain the needed precise knowledge of the *in situ* biochemical and pathological activities of the intestinal microflora. Such information will not be easy to gather because of the dynamic state of the flora. Unfortunately, *in vitro* studies with intestinal microorganisms cannot duplicate *in vivo* conditions, although work with mixed cultures under various *in vitro* conditions has been helpful.

188. Finland, M., 1955, Emergence of antibiotic-resistant bacteria. *N. Eng. J. Med.*, 353, 909–922, 969–979, 1019–1028.

A good review of floral changes in humans and animals.

189. Roine, P., Ettala, T., Raitio, A. and Vartiovaara, U., 1955, The mode of action of Aureomycin in the guinea-pig. *British J. Nutrition* 9, 181–191.

As little as a total of 0.1 mg of this antibiotic caused the test animals to stop eating and drinking almost entirely and most of them to die within one to two weeks. Daily subcutaneous injections of 0.1 to 0.3 mg aureomycin had almost no effect, but in large doses even parenteral administration proved harmful.

Bacteriological investigations showed distinct differences between the

cecal flora of control animals and of those receiving aureomycin. A striking feature in the aureomycin group was the abundance of an organism belonging to the genus *Listeria*. A direct microscopical examination of the cecal contents showed that feeding with aureomycin had considerably increased the numbers of *Listeria* and *Sarcina*, greatly reduced the lactobacilli and almost completely eliminated cells of *Oslospira*, *Fusobacterium* and *Bacillus*. It seems logical to assume that the toxic effect of aureomycin are due to the increase of the *Listeria* flora, especially as *Listeria monocytogenes* is known to occur as a parasite on a great number of wild and domestic animals, causing infections with signs mostly similar to those found in the aureomycin-fed guinea pig.

In many instances the toxic effect of aureomycin was prevented by previous (or combined) administration of penicillin, and even animals already diseased were cured with this antibiotic. Chloramphenicol also, in a few instances, produced positive results.

190. Rolfe, M. and Mayer, H., 1955, Experimentelle Studien über die Entwicklung von Merschweinchen und die Änderung ihrer Darmflora nach Verabreichung von aureomycin (Experimental Studies on the development of the guinea pig and the changes in the intestinal flora following the administration of Aureomycin). *Zbl. Vet. Med.* 2, 693-699. (*Vet. Bull.* 26, 1777, May 1956.)

For the first 14 days during the daily oral administration on aureomycin guinea pigs showed a marked loss in weight. Aureomycin also produced a significant change in the intestinal flora, which came to consist almost entirely of streptococci (*S. faecalis*) instead of the normal lactobacilli. After prolonged administration of aureomycin, lactobacilli once more predominated in the large intestine. At the same time, there was a marked increase in body weight in spite of continued dosage with antibiotic. A few animals died. Deaths coincided with maximum changes in the gut flora and when loss in body weight was not marked.

191. Hansen, M. R., Petri, L. H. and Aekert, J. E., 1954, Effects of aureomycin and vitamin B₁₂ used separately as feed supplements on resistance of chickens to *Ascaridia galli*. *Exptl. Parasit.* 2, 122-127.

Half of the chicks in each of the groups were given 100 ± 10 embryonated eggs of *A. galli*. The highest mortality rate and incidence of infestation occurred among the chicks fed only the basal ration, where there were no deaths and a much lower incidence of infestation among the chicks fed the supplemented basal ration.

Whereas Aureomycin and or vitamin B₁₂ reduced the numbers of ascarid as in the chicks, the aureomycin restricted the rate of growth of the ascarids and vitamin B₁₂ stimulated their growth. When these supplements were used together their effect on growth of the worms was nullified.

192. Jacobs, R. L., Elam, J. F. and Couch, J. R., 1955, Effect of administering antibiotics upon egg production, growth and antibiotic-resistant microorganisms. *Poultry Sci.* 34, 1232.

The basal diet contained sources of the fish and whey factors. The feeding of the antibiotics at the high level (25 to 50 mg per pound) increased egg production 10 to 19% over a seven month period, but had no effect on hatchability.

The addition of 50 mg per pound of aureomycin, terramycin, and bacitracin to a diet containing sources of the fish and whey factors increased the growth rate of New Hampshire chicks at 10 weeks over that obtained by the addition of 5 mg per pound of the antibiotic to the basal diet. A study of the fecal microflora from the hen and chick studies revealed that there was an increase in the number of antibiotic-resistant bacteria when an antibiotic was added to the diet. The feeding of the antibiotics at the low and high levels also increased the yeast cell counts and decreased the total clostridia.

193. Smith, H. W. and Crabb, W. E., 1957, The effect of the continuous administration of diets containing low levels of tetracyclines on the incidence of drug-resistant *Bacterium coli* in the feces of pigs and chickens: the sensitivity of the *Bact. coli* to other chemotherapeutic agents. *Vet. Rec.* **69**, 24-30.

A very much higher proportion of tetracycline-resistant *Bact. coli* were found in the feces of pigs and chickens which had been fed diets containing low levels of tetracyclines (4 to 30 gm/ton feed for 5 to 36 months) than were found in the feces of pigs and chickens kept on farms where these agents had never been fed.

Examination of fecal samples from pigs before and after commencing tetracycline feeding demonstrated the change that occurred from a predominantly sensitive to a predominantly resistant *Bact. coli* fecal flora. A high proportion of tetracycline-resistant *Bact. coli* were found in the feces of piglets whose mothers had been fed tetracyclines.

194. Clawson, A. J., Sheffy, B. E. and Reid, J. T., 1955, Some effects of feeding chlortetracycline upon the carcass characteristics and the body composition of swine and a scheme for the resolution of the body composition. *J. Animal Sci.* **14**, 1122-1132.

As the result of a study of data published on the body composition of 127 pigs, it was found that the water and fat contents are highly correlated (-0.98) and that the relationship between these two characteristics allows an accurate prediction of the fat content from a knowledge of the water content. The composition of the fat-free body was found to be variable; the fat-free matter of the body varies in water content inversely with the original fat content of the whole animal. The protein and ash contents of the fat-free, dry body were found to be remarkably constant (83.1 ± 1.6 and $16.9 \pm 1.6\%$, respectively).

195. Kropf, D. H., Lewis, P. K., Jr., Grummer, R. H., Bray, R. W. and Phillips, P. H., 1955, The effect of protein level, protein quality and chlortetracycline (Aureomycin) upon growth, feed efficiency and carcass composition of swine. *J. Animal Sci.* **14**, 1220-1231.

No treatment differences were observed in dressing percentage, viscera weight, kidney weight, leaf fat weight; percentage, yield of picnic shoulder, loin or total lean cuts; percentage of bones ham, percentage of total fat cut, carcass length or eye muscle area at the 10th and 13th ribs. However, liver weight tended to increase with an increase in feed protein level. Animals fed antibiotic differed significantly from those of the other lots in the following characters: percentage yield of Boston butt and picnic shoulder (greater); percentage fat back and clear plate (less) and thickness of fat back.

196. Robison, W. L., Kinkle, L. E. and Cahill, V. R., 1956, The use of an antibiotic in rations for hogs. Ohio Agric. Exper. Sta. Res. Bull. 769.

The carcasses from hogs fed low-protein rations with and without an antibiotic in them yielded 48.8 and 48.4% in lean cuts and 27.1 and 27.5% in fat for lard, respectively. The average back-fat thickness of the carcasses from the two groups of hogs as named was 1.81 and 1.92 inches.

The carcasses from hogs fed standard protein rations with and without an antibiotic in them yielded 50.3 and 50.6% in lean cuts, including the lean trimmings, and 25.2 and 25.3% in fat for lard, respectively. The average back-fat thickness of the carcasses from hogs as named was 1.76 and 1.79 inches, respectively.

197. Bowland, J. P., Beacom, S. E. and McElroy, L. W., 1951, Animal protein factor and antibiotic supplementation of small grain rations for swine. *J. Animal Sci.* 10, 629-637.

Pigs receiving an APF supplement without antibiotic gained slightly more rapidly and efficiently than did the controls.

198. Merck Service Bulletin, Procaine penicillin in animal nutrition. Merck and Company, Inc. Pub. Rahway 1956.

199. Beeson, W. M., 1952, Effect of antibiotics on the fatness of hogs. *J. Am. Vet. M. A.* 121, 95.

Two tests have indicated that in 224 lb hogs the fat thickness of the back is increased from 1.72 inch to 2.03 inch by antibiotic feeding. This suggests that part of the extra weight secured by antibiotics feeding may be due to greater depositions of fat.

200. Perry, T. W., Beeson, W. M. and Vosteen, B. W., 1953, The effect of an antibiotic or a surfactant on the growth and carcass composition of swine. *J. Animal Sci.* 12, 310-315.

Significantly increased growth rate was obtained by feeding 7.5 mg Aureomycin hydrochloride per pound of total ration ($P < 0.01$) or 0.26% alkyl benzene sulfonate (approached $P < 0.05$) in the ration of growing fattening swine.

Carcasses from antibiotic-fed hogs contained significantly more ($P < 0.01$) fat and significantly less ($P < 0.01$) protein and water than carcasses from the control animals. Carcasses from surfactant-fed hogs likewise contain significantly more ($P < 0.05$) fat and significantly less ($P < 0.05$) protein and water than carcasses from the control animals.

201. Catron, D. V., Bennison, R. W., Maddock, H. M., Ashton, G. C. and Homeyer, P. G., 1953, Effects of certain antibiotics and vitamin B₁₂ on pantothenic acid requirements of growing-fattening swine. *J. Animal Sci.* 12, 51-61.

Antibiotic fed pigs consumed 28% more water and 13% more feed while gaining 27.5% faster than the pigs fed no antibiotics. Carcass studies revealed no differences in back fat, body length or tissue moisture content between the antibiotic and nonantibiotic fed pigs.

202. Wilson, G. D., Burnside, J. E., Bray, R. W., Phillips, P. H. and Grummer, R. H., 1953, Pork carcass value as affected by protein level and supplementation with aureomycin and vitamin B₁₂. *J. Animal Sci.* 12, 291-296.

On a ration averaging 18% protein, the addition of 20 mcg of vitamin B₁₂ and 20 mg of aureomycin alone or in combination did not significantly increase the percentage of lean cuts, dressing percentage, back fat depth or length of carcass. Within the medium protein lots an increase in the leanness of the carcasses resulted from all treatments, the combination for aureomycin and B₁₂ resulting in as high a percentage of lean cuts as the high-protein basal ration. Supplementation of the low-protein ration resulted in an increase in the percentage of lean cuts in each of the three lots, the increase being somewhat greater than in the medium protein groups with similar supplements. Fat deposition was not increased when the addition of vitamin B₁₂ and aureomycin accelerated gains.

203. Clark, H. E., Harrison, D. L., Soule, R. P., Jr. and Richardson, D., 1955, The nutritive value of proteins of muscle from hogs fed diets supplemented with aureomycin or terramycin hydrochloride. *J. Nutrition* **56**, 61-66.

The nutritive value of proteins of pork was studied when hogs were fed (1) a basal ration adequate for growth; (2) the basal ration supplemented with aureomycin hydrochloride; or (3) the basal ration supplemented with terramycin hydrochloride. Growth and nitrogen balance of weanling rats that were offered 1.6% of nitrogen served as criteria. Inclusion of 10 mg of aureomycin or terramycin per pound of ration fed to hogs did not stimulate the growth of rats, but nitrogen balance, expressed in terms of surface area, was significantly higher ($P < 0.05$) when pork representing the basal ration was fed than when meat from hogs receiving either of the antibiotics was offered. The nutritive value of pork proteins was similar to that of beef for the growing rat.

204. Kelly, R. F., Bray, R. W. and Phillips, P. H., 1957, The influence of chlortetracycline supplementation of the ration on distribution, quantity and quality of fat deposited in swine. I. Metabolic effects in relation to carcass composition. *J. Animal Sci.* **16**, 74-83.

The blood fat levels for the chlortetracycline-supplemented lots were lower (not significantly) than the blood fat levels of the controls. The absorptive blood fat levels of 205 lb groups were significantly higher than the levels of the 165 and 85 lb groups. The iodine number of the lean and back-fat from barrows were lower (near 5% and 1% respectively) due to chlortetracycline supplementation.

The average back-fat thickness of the males was significantly (5%) increased as a result of chlortetracycline supplementation. This finding is in agreement with the trend toward greater carcass fatness as expressed in other physical and chemical measurements.

205. Dymaza, A., Boucher, R. V. and Callenbach, E. W., 1953, The influence of antibiotic supplementation on certain physical and chemical characteristics of turkey poults. *Poultry Sci.* **32**, 989-993.

Chemical analysis showed that the growth increase attributed to terramycin had little relation to carcass composition, with the possible exception of an increase in dry matter content. The ether extract content varied considerably between individual samples, and was slightly but not significantly higher in carcasses from the terramycin fed group. No group differences were apparent in carcass protein or ash content.

The overall effect of antibiotic supplementation appeared to be production of larger birds of normal composition.

206. Saxena, H. C., Blaylock, L. G., Stadelman, W. F., Carver, J. S. and McGinnis, J., 1953, Effect of penicillin on growth, feed efficiency and fattening of turkeys. *Poultry Sci.* **32**, 721-23.

207. Jukes, H. G., Hill, D. C. and Branion, H. D., 1957. Effect of penicillin on the carcass composition of the chicken. *Poultry Sci.* **36**, 423-425.

A higher percentage body fat of the chickens receiving penicillin (10 ppm and 200 ppm) was recorded in both experiments, but only in one experiment was this difference statistically significant. A small reduction in percentage of moisture, protein and ash was also noted when penicillin was fed, but the differences were not statistically significant. The greater average weight of birds receiving penicillin was not accounted for solely by an increase in fat.

208. Ozawa, E., 1955, Studies on growth promotion by antibiotics. I. Effects of chlortetracycline on growth. *Jour. Antibiotics* **8**, 205-211. (*Biol. Abstr.* **31**, 4034, Feb. 1957.)

Between mice kept on a basal diet and those on a diet supplemented with chlortetracycline, there was little difference in bone weight and in tissue water; tissue protein was somewhat higher and neutral fat significantly higher in mice on the supplemented diet. Mice kept on a high fat diet supplemented with chlortetracycline had considerably less fat deposition in the liver than did those animals maintained on the same diet without the supplement. Mice kept for several weeks on a minimal diet began to gain weight rapidly once the diet was supplemented with chlortetracycline.

209. Owen, F. G., Voelker, H. H., Jacobson, N. L. and Allen, R. S., 1955, The comparative effects of various antibiotics and an arsenical upon the growth, health, and certain blood constituents of dairy cows. *J. Dairy Sci.* **38**, 891-900.

Blood cell counts (erythrocytes, leukocytes and differential leukocytes), hemoglobin, plasma fat and fecal pH appeared to be unaffected by antibiotic or arsenical supplementation.

210. Squibb, R. L., Salazar, R., Guzman, M. and Scrimshaw, N. S., 1953, Effect of Aureomycin and vitamins on growth and blood constituents of pigs fed corn and banana ration. *J. Animal Sci.* **12**, 297-303.

Aureomycin did not have any apparent effect on serum proteins, riboflavin, ascorbic acid, carotenoids, vitamin A, tocopherols, red-cell count, hemoglobin and hematocrit in the blood of young growing pigs.

The increase in alkaline phosphatase values observed in pigs fed either corn or bananas seemed to be depressed by the addition of Aureomycin to the pigs' diet.

211. Shefchik, B. E., Acevedo, G., Grummer, R. H., Phillips, P. H. and Bohstedt, G., 1950, Comparison of growth responses to streptomycin, aureomycin and crude APF, alone and in combination with B₁₂ on 2-day old pigs using a "synthetic" milk. *J. Animal Sci.* **9**, 667.

Results indicate that aureomycin gave a greater growth response than did streptomycin. A combination of the two antibiotics gave the best results. Hemoglobin data did not show any consistent differences between lots.

212. Lassiter, C. A., Denton, T. W. and Rust, J. W., 1955, The effects of chlortetracycline and Ethomid C/15 on growth, apparent digestibility and blood levels of urea and total non-protein nitrogen in young dairy calves. *J. Dairy Sci.* **14**, 760-768.

The control calves gained 0.99 lb daily whereas those fed aureomycin gained 1.26, and those fed Ethomid C/15 1.16 lb daily. These differences were statistically significant. An improvement in feed efficiency and a lowering of the incidence of scours were observed with the aureomycin-supplemented calves.

213. Luther, H. G., Reynolds, W. M., McHahan, J. R. and Kersey, R. C., 1953, Antibiotic carry-over in tissues of livestock. *Antibiotics Ann.* **1953-54**. Proc. Symposium on Antibiotics, Washington D. C., pp. 416-420.

Withdrawal of antibiotics from the feed has a pronounced effect on disappearance of residual antibiotic from the tissues of poultry and swine. In three days after withdrawal, all residual activity has disappeared.

214. Smith, Q. T. and Allen, R. S., 1953, B-vitamin levels in the blood of young dairy calves fed a milk replacement diet with and without aureomycin. *J. Dairy Sci.* **36**, 593.

The data indicate that the antibiotic had no significant effect on the blood levels of these B vitamins. Moreover, no significant differences between male and female blood B-vitamin levels were noted.

215. Catron, D. V., Lane, M. D., Quinn, L. Y., Ashton, G. C. and Maddock, H. M., 1953, Mode of action of antibiotics in swine nutrition. I. Effect of feeding antibiotics on blood glucose. *Antibiotics and Chemother.* **3**, 571-577.

The animals fed the basal ration gained 1.24 lb per day, and those fed the ration containing Aureomycin gained 1.35 lb per day. The feed required per pound of gain were 2.79 and 2.69 respectively.

The normal blood glucose values again appear to be higher for the pigs fed the antibiotic. The antibiotic-fed pigs also showed a greater increase in blood glucose 15 minutes after glucose administration than the basal-fed animals.

216. Vandersall, J. H., Hibbs, J. W. and Conrad, H. R., 1956, The influence of chlortetracycline on whole blood, plasma and corpuscle glucose relationships in calves fed high roughage pellets. *J. Dairy Sci.* **39**, 929.

The chlortetracycline-fed calves maintained a consistently higher whole-blood glucose level. This higher level was found to be the result of increased plasma glucose.

217. Bogdonoff, P. D., Jr. and Shaffner, C. S., 1954, Antibiotics and calcium metabolism. *Poultry Sci.* **33**, 1044.

Bacitracin, chlortetracycline, oxytetracycline and penicillin increased plasma calcium in chicks.

218. Voelker, H. H., Jacobson, N. L. and Allen, R. S., 1955, Relationship of antibiotic feeding and the rate of growth to blood reducing sugar levels and glucose absorption in dairy calves. *Antibiotics and Chemother.* **5**, 224-231.

Reducing sugar levels in the blood following oral administration of glucose were significantly higher in calves fed 200 mg of chlortetracycline

daily (except when fasted) than in control animals. The mean weight gain of 9 calves fed the antibiotic was approximately 19% greater than the mean gain of the corresponding controls.

219. Voekler, H., Jacobson, N. L. and Allen, R. S., 1952, Effect of antibiotic feeding on blood glucose levels of young dairy cattle. *J. Animal Sci.* 11, 779.

Blood glucose values of animals fed 200–240 mg aureomycin hydrochloride (in Aurofac 2A) per calf daily for a considerable period of time were not significantly different from those of animals receiving no antibiotics.

220. Slanetz, C. A., The influence of antibiotics on antibody production, 1953. *Antibiotics and Chemother.* 3, 629–633.

Albino rats and albino mice were maintained on a pulverized standard commercial pelleted diet supplemented or not with 0.1% of antibiotics (aureomycin, oxytetracycline, chloramphenicol). Short-term feeding of antibiotics (for two days prior to antigen administration) resulted in higher antibody titers than in the controls fed the stock diet alone. Prolonged antibiotic feeding (for two weeks prior to antigen administration) appeared to interfere with antibody production.

- 220a. Glick, B., 1958, The effect of procaine penicillin on the white blood cells of chickens. *Poultry Sci.* 37, 78–81.

The antibiotic at 100 gm/ton increased the body weight, the total white cell count and the lymphocyte count. He suggested penicillin may act directly on the white cells or may activate a 'leukocytic factor.'

- 220b. Wostmann, B. S. and Gordon, H. S., 1958, Electrophoretic studies on serum proteins of young germfree, conventional and antibiotic treated conventional chickens. *Proc. Soc. Exptl. Biol. and Med.* 97, 832–835.

Antibiotics fed to conventional chicks reduced the size of the ileocecal lymph nodes in chicks and after 3 months the circulating gamma globulins was reduced. No growth difference was noted.

221. Carpenter, J. W. and Pearson, A. M., Wallace, H. D., Jack, F. H. and Milicevic, M., 1953, The content of B-complex vitamins in the tissues of pigs fed various levels of protein with and without aureomycin. *J. Animal Sci.* 12, 900.

222. Shirley, R. L., Wallace, H. D. and Davis, G. K., 1954, Effect of dietary aureomycin and different levels of protein on several phosphorus and nitrogen compounds in hams. *J. Agric. Food and Chem.* 2, 830–832.

Statistical analysis of the data showed no significant differences in the acid soluble and nucleic acid phosphorus, ammoniacal and nucleic acid nitrogen, and total solids values obtained for the six dietary groups. The lipid and phosphoprotein phosphorus and the protein and phosphoprotein nitrogen showed variances between the dietary groups at the 0.05% level of significance. The lipid phosphorus variation was due to interaction of the protein and the aureomycin. The antibiotic increased the phosphoprotein phosphorus in the intermediate and high-protein rations, but had no effect in the low protein ration. The higher levels of dietary protein resulted in an increase.

223. Ozawa, E., 1956, Studies on growth promotion by antibiotics. IV. Effect of chlortetracycline (Aureomycin) on the endocrine organs. *J. Antibiotics (Tokyo)* 9, (2) 71–74. (*Biol. Abstr.* 31, 7201, March 1957.)

Three control rabbits were maintained on a basal diet and 3 on a diet supplemented with 20 mg chlortetracycline per day; after 3 weeks the animals were killed by air embolism and tissue studies were made on muscle. The chlortetracycline-treated animals exhibited increased numbers of eosinophils and decreased numbers of main cells in the anterior lobe of the hypophysis, and increased storage of liver and muscle glycogen, no changes were noted in the adrenals or pancreas. Adrenal cortex function of guinea pigs was investigated by study of the urine before and after supplementation of their diet with chlortetracycline. Administration of chlortetracycline increased excretion of 17-ketosteroid, particularly in animals that had been in a state of malnutrition. The increase in adrenal cortex function correlates with the increased deposition of glycogen in muscle and liver of treated rabbits.

224. Luckey, T. D., 1959, The nutrition and biochemistry of germfree chicks. *Ann. N. Y. Acad. Sci.* **78**. In press.

225. Sunde, M. L., Waibel, P. E., Cravens, W. W. and Elvehjem, C. A., 1951, A relationship between antibiotics, vitamin B₁₂ and choline and methionine in chick growth. *Poultry Sci.* **30**, 668-671.

Since increased vitamin B₁₂ was found in the livers of chicks fed streptomycin, this drug is sparing the vitamin B₁₂ which indirectly spares the methionine and choline need of the chick.

226. Barber, R. S., Braude, R., Kon, S. K. and Mitchell, K. G., 1952, Effect of supplementing an all-vegetable pig-fattening meal with antibiotics. *Chemistry and Industry*, **29**, 713.

The results obtained indicate that the addition of antibiotics to vegetable-protein diet significantly improved growth rate and feed efficiency.

After slaughter the amounts of B₁₂ and vitamins in the livers were determined. B₁₂ levels were decidedly higher in pigs given the aureomycin mash and the streptomycin residue + penicillin. Highest liver stores of vitamin A were found in pigs given aureomycin either with or without supplementary B₁₂.

227. Bentley, O. G. and Hershberger, T. V., 1954, The effect of antibiotics on hatchability of hens' eggs and progeny growth performance. *Poultry Sci.* **33**, 641-648.

Feeding antibiotics (bacitracin, terramycin, aureomycin HCl, or procaine penicillin) to hens did not consistently improve hatchability of fertile eggs. However, in one experiment there was some increase in hatchability with either bacitracin or aureomycin HCl, both in the presence and absence of supplementary vitamin B₁₂.

228. Coates, M. E., Harrison, G. F., Kon, S. K., Porter, J. W. G. and Thompson, S. Y., 1952, Antibiotics in chick nutrition and vitamin A metabolism. *Chem. and Indust.* **55**, 297.

Penicillin fed chicks had improved conversion of carotene into vitamin A in the intestinal wall and higher concentration of vitamin A in the liver than control chicks.

229. Muller, A., 1957, Feeding antibiotics and their effect on vitamins, *Socialist. Zemedelstvi, Za* **7**, 582-485. (*Chem. Abstr.* **51**, 12257, Aug. 25, 1957.)

A summary of the effects of addition of antibiotics to animal feeds. By addition of 10 mg of chlortetracycline or 5 mg of penicillin to 1 kg of

feed vitamin A in the liver of chicks was increased 1.6 and 1.8%, carotene 2.0 and 2.4% and carotenoids 1.6 and 1.7% respectively. By addition of 10 mg of chlortetracycline to 1 kg of rat ration containing no vitamin B₁₂ but containing a large amount of methionine, the lack of vitamin B₁₂ had no harmful effects. Penicillin increased the synthesis of vitamin B₁ in the digestive system. Fifty mg of chlortetracycline, penicillin, oxytetracycline and streptomycin were added to 1 kg of ration containing limited amounts of vitamin B₁ and B₂ and pantothenic acid. All antibiotics favored the growth with limited amounts of vitamin B₂ and pantothenic acid but only chlortetracycline and oxytetracycline favored the growth with limited amounts of vitamin B₁. In the chicks, chlortetracycline, penicillin and bacitracin, with the help of coliform bacteria, synthesized folic acid. Chlortetracycline increased the utilization of Mn and of nicotinamide and prevented perosis in the chicks. A favorable effect is shown of the antibiotics on vitamins including vitamin K.

231. DiRaimondo, F., Mannino, N. and Trinchese, L., 1952, Effetti collaterali di antibiotici nel ratto: contenuto del fegato in vitamine del complesso B equadro isologico dopo prolungato trattamento (Side effects of antibiotics on the rat: content of the liver in vitamins of the B complex and histologic findings after prolonged treatment). *Internat. Zschr. Vitaminforsch.* **24**, 294-301. (*Chem. Abstr.* **47**, 3419, April 10, 1953.)

Prolonged administration of terramycin, aureomycin and chloramphenicol orally, and of chloramphenicol intramuscularly to albino rats produced marked decreases (determined microbiologically) in the nicotinamide, folic acid and cobalamin (vitamin B₁₂) content of the liver, and a decrease in its pyridoxine content. The effect was increased by feeding a vitamin-free ration.

232. Brown, J. A., Robblee, A. R. and Clandinin, D. R., 1953, The use of penicillin breeding ration. *Poultry Sci.* **32**, 576-578.

Penicillin had no effect on rate of production, feed efficiency or average body weight during a trial lasting for 336 days. Fertility and hatchability of eggs produced were likewise not affected by the addition of penicillin to the rations.

Penicillin supplementation had no effect on the liver storage of riboflavin.

233. Sutherland, D. A., Mann, J. D., Giges, B. and Seligson, D., 1951, Effect of Aureomycin on liver fat and liver function. *Proc. Soc. Exptl. Biol. and Med.* **77**, 458-459.

This drug was shown to have no effect on liver fat (biopsy) or liver function in male human subject, rats and dogs.

234. Conrad, H. R., Hibbs, J. W. and Pounden, W. D., 1952. Rumen synthesis of some of the B-complex vitamins in calves raised on the high roughage system. *J. Animal Sci.* **11**, 759.

In a study of the synthesis of some B-complex vitamins in the rumen of young dairy calves fed on different variations of the high-roughage system it was found that thiamine and riboflavin levels in the rumen juice were not affected by feeding 20 mg of aureomycin (Aurofac-2A) per pound of dry feed.

235. Abbott, O. J., Bird, H. R. and Cravens, W. W., 1954, Effects of dietary arsenilic acid on chicks. *Poultry Sci.* **33**, 1245-1253.

Arsenilic acid reduced B₁ requirements.

236. Ross, E. and Yacowitz, H., 1952, Effects of Penicillin on vitamin D requirements for growth and bone calcification. *Poultry Sci.* **31**, 1933.

Penicillin did not affect growth of chicks fed a diet low in vitamin D but it did increase the bone ash (calcium absorption).

237. Tillman, A. D. and MacVicar, R., 1956, The effect of chlortetracycline upon digestion of ration components, retention of nitrogen and volume of urine excreted by sheep with observations on rectal temperatures. *J. Animal Sci.* **15**, 211-217.

Three digestibility trials involving 58 sheep were conducted to study the effect of chlortetracycline upon the digestibility of ration components. It was found that a level of 11.8 mg of chlortetracycline per 100 lb body weight or 11 mcg per gram of feed had no effect upon ration digestibility. When the level was increased to 15.4 mg per 100 lb body weight or 16.7 mcg per gram of feed, there was significant reduction in the digestibility of dry matter, crude protein, crude fiber, nitrogen-free extract and energy.

238. Tillman, A. D., MacVicar, R. W., 1953, The effect of aureomycin upon growth, digestion of ration constituents, rectal temperatures and urine volume of sheep. *J. Animal Sci.* **12**, 955-956.

The average rectal temperatures were 39.93°C and 39.77°C for the basal and aureomycin groups, respectively. The average daily excretion of urine was 898 and 1,136 gm per sheep/day for the basal and aureomycin groups respectively.

239. Coniglio, G. J. and Bell, E. J., 1957, The effect of antibiotics upon intestinal and hepatic lipogenesis. *J. Biol. Chem.* **226**, 805-811.

Rats fed aureomycin incorporate more acetate into hepatic fatty acids than do control rats (C¹⁴ study).

240. Coates, M. E., 1953, The mode of action of antibiotics in animal nutrition. *Chem. and Industry* **50**, 1333-1335.

Addition of penicillin to the diet of chicks maintained in newly disinfected quarters did not result in an improved growth rate.

Chicks kept in new small isolation units showed no growth response to penicillin over a 12- to 14-day test period. However, when a sample of whole gut contents from chicks kept on "infected" premises was added to the ration, a severe growth depression resulted which was counteracted, though not always completely so, by penicillin. A similar preparation from "uninfected" chicks did not cause weight depression and neither did the "infected" gut contents after autoclaving.

The gut of chicks receiving antibiotics was found to be distinctly lighter in weight than that of untreated controls. The weight difference was not due to length of the gut nor to altered fat or water content. Histological examination revealed no differences between the heavier control gut and the lighter penicillin-treated gut.

The author finds the hypothesis that the "infection" attacks the gut, directly decreasing the efficiency and absorptive powers, thereby resulting in small deficiencies, of many vitamins, is in accordance with their findings that a thicker gut wall and a less efficient conversion of carotene occur in chicks not given antibiotics.

241. High, E. G., 1955, Influence of aureomycin, penicillin, and vitamin B₁₂ on metabolism of carotene and vitamin A in rat. *Fed. Proc.* **14**, 437.

Chlortetracycline fed rats stored more vitamin A, converted from carotene, in liver and kidney tissue than did control rats.

242. Vonk, M. A. A., McElroy, L. W., Bowland, J. P. and Berg, R. T., 1957, The effect of ingested chlortetracycline on some hydrolases and organs associated with the digestive process in growing pigs. 2. Pancreas—dry weight, fat content, protease and amylase activity. *Canad. J. Biochem. and Physiol.* **35**, 187–193. (*Nutrition Abstr. and Rev.* **27**, 4991 Oct. 1957.)

Chlortetracycline increased body weight gain and efficiency of feed utilization. It significantly increased pancreas dry weight, total protease, total amylase and amylase per gram dry matter. The difference remained significant only for amylase after adjustment for body weight by covariance. There was no significant difference in the crude fat content of the pancreas attributable to chlortetracycline.

243. Thayer, R. H., 1956, Role of antibiotics in modifying the energy, vitamin and protein requirements of chicks. *Dissert. Abstr.* **16**, 424. (*Abstr. J. Sc. Food and Agric.* **7**, 1191).

Penicillin increases the efficiency with which proteins, vitamins and energy are utilized by the growing chick, increases the per cent of dietary nitrogen absorbed, reduces the per cent of absorbed dietary nitrogen excreted as urinary nitrogen and increases the rate of enzyme activity per gram of intestinal weight.

244. Melnykowycz, J. and Johansson, K. R., 1955, Formation of amines by intestinal microorganisms and the influence of chlortetracycline. *J. Expt. Med.* **101**, 507–517.

235. Hill, E. G. and Larson, N. L., 1957, Effects of products obtained from streptomyces aureofaciens fermentation on growth and reproduction of swine. *J. Sc. Food and Agric.* **8**, 1–188.

Supplementing the ration of pregnant gilts with chlortetracycline (200 ppm) for 80 days resulted in a significant increase in the viability of baby pigs farrowed naturally (from 55 to 78%) or by hysterectomy (from 76 to 94%; birth wt., no. of pigs per litter and number of corpora lutea recovered as live pigs were unaffected). Placental transfer of the antibiotic did not occur. Supplementation of the ration of young pigs decreased the concentration of amines in the ileum.

246. Tomlin, D. C., Wallace, H. D., Combs, G. E., Jr. and Alsmeyer, W. L., 1956, Studies on the palatability of antibiotics for swine. *J. Animal Sci.* **15**, 1230.

Each pen of pigs in all experiments was offered in separate feeders the various antibiotics. Feeders were rotated at weekly intervals to overcome position preference. The following figures give the relative acceptability of the various antibiotics for the three experiments, respectively, expressed as a per cent of the total feed consumed: chlortetracycline—49.6, 66.2, 64.8; oxytetracycline—20.4, 13.2, 9.9; penicillin V—29.6, 14.2, 3.2; erythromycin—0.6, 0.0, 0.0, and no antibiotic—this treatment was not offered in the first experiment, 6.4, 22.1. These results indicate that weanling pigs have a preference for chlortetracycline.

strongly dislike erythromycin and have no particular likes or dislikes for oxytetracycline and penicillin V.

247. Tomlin, D. C., Wallace, H. D. and Combs, G. E., Jr., 1958, The influence of certain antibiotics on the palatability of swine rations. *J. Animal Sci.* **17**, 42-47.

Chlortetracycline in rations at 40 gm/ton enhanced the palatability of swine rations and was much preferred in free choice over rations containing oxytetracycline or penicillin V which did not affect palatability. Erythromycin decreased palatability markedly.

248. Fevrier, R. and Vachel, J. P., 1955, Les antibiotiques et al croissance du porc. 2. Adjonction de penicilline et d'aureomycine à un régime dépourvu de protéines animales. *Ann. Zootech.* **4**, 136-138. (*Nutrition Abstr. and Rev.* **26**, 2542. 1956.)

Growth rate and efficiency from 50 to 200 lb live weight were improved by aureomycin (chlortetracycline) plus vitamin B₁₂ and by procaine penicillin added to a ration containing only vegetable protein. The greatest stimulation was in the aureomycin group and was slightly more from 100 to 200 lb live weight than from 70 to 100 lb. There was no effect on carcass quality or the capacity of the cecum. The antibiotics improved appetite.

249. Hare, J. H., Soule, R. P., Jr. and Zucker, H., 1957, Palatability of broad-spectrum antibiotics for swine. *J. Animal Sci.* **16**, 1081.

In one replicate pen, total consumption of oxytetracycline-containing feed was 2,375 lb and of chlortetracycline-containing feed 1,648 lb. In the other replicate, these quantities were 954 and 2,925 lb respectively. The study was then extended to practical conditions, with the use of feed grade products supplying 40 gm of the respective antibiotics per ton of feed, the animals being permitted a choice of rations. Feed consumption of oxytetracycline- and chlortetracycline-containing feeds were, respectively, 2,754 and 932 lb in Replicate 1 and 2,668 and 726 lb in Replicate 2. Daily rates of gain were improved by either the crystalline or feed grade antibiotic supplements.

250. Dowe, T. W., Matsushima, J. and Arthaud, V. H., 1957, The effects of corn treated with fungicides upon the performance of fattening steers. *J. Animal Sci.* **16**, 93-99.

Aroson decreased food consumption—Orthocide did not. No change in gain was seen.

251. Slinger, S. J. and Pepper, W. F., 1954, Effect of penicillin on the growth and feed consumption of turkey poults. *Poultry Sci.* **33**, 746-753.

The results obtained indicated that, under the conditions employed, the mode of action of penicillin in stimulating the growth of turkey poults could be explained largely, though not entirely, on the basis of an increased feed consumption per unit of body weight which occurred during the first week of life.

252. Stokstad, E. L. R., Jukes, T. H. and Williams, W. L., 1953, The growth-promoting effect of aureomycin on various types of diet. *Poultry Sci.* **32**, 1054-1058.

The response to aureomycin has been determined with various types of carbohydrates in the basal diet and has been found to be greatest with sucrose. The time of food passage on a sucrose diet was in-

creased by aureomycin but was not changed on starch and glucose diets. Growth responses to both yeast and aureomycin were obtained on diets containing approximately 20% protein.

253. Peterson, G. E. and Johansson, K. R., 1953, Effects of oral and parenteral administration of degraded and active antibiotics to rats fed a vitamin B₁₂ deficient ration. I. Growth and intestinal microflora. *Antibiotics Ann.* 1953-54. Proc. Symposium on Antibiotics, Washington D. C., pp. 386-394.

The oral administration of oxytetracycline, chlortetracycline and penicillin stimulated the growth and the food efficiency of rats fed a diet deficient in vitamin B₁₂. Likewise, the parenteral administration of chlortetracycline, bacitracin and penicillin promoted growth and food efficiency. Alkali treatment and heat treatment destroyed the growth enhancing property of chlortetracycline and penicillin respectively, toward these animals.

Regardless of the route administered, these antibiotics, including the inactivated agents, provoked marked changes in the fecal, cecal and ileal microflora. Since antibiotic-induced modifications of the intestinal microflora were not always accompanied by a growth effect in the host, it was suggested that theories implicating the intestinal flora as the sole factor in the growth-promoting characteristics of antibiotics are not beyond suspicion.

254. Hogue, D. E., Warner, R. G., Loosli, J. K. and Grippin, C. H., 1957, Comparison of antibiotics for dairy calves on two levels of milk feeding. *J. Dairy Sci.* 40, 1072-1078.

The level of milk feeding had a definite effect on the growth of the calves. Calves fed 350 lb of milk grew faster for the first seven weeks than those fed 175 lb of milk. In most criteria observed, the antibiotics tended to minimize this difference by increasing the performance of the lower milk fed group. The antibiotics were effective on the 350 lb milk group only in the presence of digestive disorders.

It is suggested that the effects of the antibiotics on incidence of diarrhea and average daily gain are, at least in part, independent and that the antibiotics may have a milk-sparing effect in allowing the use of lower milk feeding regimes.

- 254a. Pepper, W. F., Slinger, S. J. and Motzok, I., 1951, The effect of aureomycin on the interrelationship between manganese and salt in chicks. Abstr. Papers Presented at 40th Ann. Mtg. of Poultry Sci. Assoc.

Alterations in growth due to antibiotic were reflected in differences in water consumption.

Aureomycin lowered the amount of manganese required for growth and perosis prevention suggesting that the effect of the mineral may be mediated through the intestinal flora.

255. Weber, E. M., Luther, H. G. and Reynolds, W. M., 1952, Antibiotics as animal growth stimulants. World Health Organization Monograph. Series No. 10. First International Symposium on Chemical Microbiology, pp. 149-161.

Chicks in a 4 week experiment were found to imbibe almost twice as much water when fed different antibiotics. They postulate the proteins of the intestinal wall would take up more water with its dissolved

crystalloids, which is an increased nutrient absorption from the intestinal tract.

	Control	Terramycin
Ave. 4 week consumption	620 ml	1186 ml
1st week consumption	53	149
2nd week consumption	260	397
3rd week consumption	134	324
4th week consumption	173	315

256. Braude, R. and Johnson, B. C., 1953, Effect of aureomycin on nitrogen and water metabolism in growing pigs. *J. Nutrition* **49**, 505-512.

No effect on nitrogen retention was observed, even though there was an increase in efficiency of food utilization and considerably higher urinary excretion (urinary water losses) was observed with animals receiving the antibiotic.

257. Slinger, S. J. and Pepper, W. F., 1955, Effect of water intake on the growth response of poult and chicks to penicillin. *J. Nutrition* **57**, 319-328.

The effect of water intake on the growth response of turkey poult to penicillin was studied under conditions of both free choice and equated feed intake. Regardless of the method of feeding, the addition of penicillin (25 mg procaine penicillin/kg feed) resulted in about the same growth response in poult whose water intake was restricted as in those receiving water *ad libitum*. The interaction between penicillin and method of watering was not statistically significant.

In general, chicks and poult fed penicillin exhibited less desire for water than the controls. The results indicate that increased water intake is not necessary to elicit the penicillin growth response and that penicillin tends to have a "sparing" effect on the water requirement of these species.

258. Lindblad, G. S., Slinger, S. J. and Motzok, I., 1954, Effect of aureomycin on the calcium and phosphorus requirements of chicks and poult. *Poultry Sci.* **33**, 482-491.

Aureomycin improved chick and poult weight in all cases. The more inadequate the ration in calcium and phosphorus, the greater was the percentage increase in weight due to the antibiotic. Aureomycin seemed to increase the requirement of male chicks for phosphorus.

The calcium and phosphorus requirements of female poult were not appreciably altered by the addition of aureomycin to the diet. However, a statistically significant interaction was shown between phosphorus and sex, suggesting that male poult have a higher requirement for inorganic phosphorus than the generally recommended levels of 0.4%. This may be due to the fact that the interaction between antibiotic and sex was highly significant ($P = 0.01$) in favor of the male poult.

259. Brown, W. O., 1957, The effect of dietary penicillin on calcium and nitrogen retention in chicks on a low mineral diet. *J. Sc. Food and Agric.* **8**, 279-282.

Chicks were raised on a low calcium diet with added penicillin (20 mg/kg of food). It was found that a significant increase in calcium

retention in the body is brought about by the addition of penicillin to the diet. The addition of penicillin to chick diets low in calcium results in increased absorption of calcium and an overall increase in weight.

260. Migicovsky, B. B., Nielson, A. M., Gluck, M. and Burgess, R., 1951, Penicillin and calcium absorption. *Arch. Biochem. Biophys.* **34**, 479-481.

Penicillin treated chicks showed an increase uptake of Ca.⁴⁵

261. Ross, E. and Yacowitz, H., 1954, Effect of penicillin on growth and bone ash of chicks fed different levels of vitamin D and phosphorus, *Poultry Sci.* **33**, 262-265.

The addition of 2.5 gm of crystalline procaine penicillin G per ton of an all-plant protein basal ration significantly increased bone ash in chicks over a three-week experimental period. Penicillin had no effect on the vitamin D requirement for growth nor did it stimulate growth in chicks fed vitamin D deficient, low phosphorus ration.

262. Gabuten, A. R. and Shaffner, C. S., 1954, A study of the physiological mechanisms affecting specific gravity of chicken eggs. *Poultry Sci.* **33**, 47-53.

The calcium level in the blood serum and the shell-breaking strength of the eggs were higher in the penicillin-fed birds than in the controls.

263. Bush, L. J., Jacobson, N. L. and Allen, R. S., 1957, Effect of chlortetracycline on the metabolism of dairy calves with particular reference to calcium utilization. *J. Animal Sci.* **16**, 1091.

Blood levels of Ca⁴⁵ (expressed as per cent of the single administered dose either per 100 ml blood or in estimated total blood) were higher in the control group during the 24 hours following administration. Radioautographs were made from a tibia and femur of each calf following sacrifice at 16 weeks of age. During the 16-week period the average weight gain was 165.5 lb for the calves receiving chlortetracycline and 146.1 lb for the controls. The gain in height at withers for the first 16 weeks was 11.9 and 10.3 cm for the two groups, respectively. Feed consumption was greater for the group receiving chlortetracycline.

264. Main, H. M., 1952, A study of the mechanism of action of growth stimulants on the intestinal microflora of fowl. Thesis, Univ. of Toronto.

In general, penicillin, aureomycin and 3-nitro-4-hydroxyphenyl arsonic acid increase the weight and improve the efficiency of feed utilization in chicks.

Aureomycin improves the utilization of both manganese and niacin and reduces the incidence of perosis due to a deficiency of one or both of these nutrients.

Penicillin appears to overcome the deleterious effect of certain surfactants on chick growth. It also relieves interference caused by urea.

Several surfactants produce increases in chick weight but, in general, do not appear to improve feed efficiency to any marked extent.

Dietary penicillin, aureomycin, 3-nitro-4-hydroxyphenyl arsonic acid, certain surfactants and methionine all tend to reduce the pH of cecal contents. The most pronounced decreases appear to be due to the antibiotics.

265. Anderson, G. W., Main, H. M. and Wright, M. L., 1957, The role of chlortetracycline hydrochloride and intestinal microflora in the niacin

and manganese requirements of chicks. *Canad. J. Comp. Med. and Vet. Sc.* **21**, 336-343.

Chlortetracycline HCl enhanced the utilization of both manganese and niacin for growth promotion but did not appear to reduce the dietary requirements for either nutrient.

266. Erwin, E. S., Dyer, I. A. and Ensminger, M. E., 1955, Digestibility of steer fattening rations as affected by quality of roughage, fat, chlortetracycline (aureomycin) and stilbestrol. *J. Animal Sci.* **14**, 1201-1202.

Chlortetracycline significantly increased the digestibility of ether extract whereas stilbestrol did not significantly affect the digestibility of ether extract. Coefficients of digestibility of dry matter were significantly higher in the alfalfa rations than the straw rations.

267. Hogue, D. E., Warner, R. G., Loosli, J. K. and Grippin, C. H., 1955, Digestibility and nitrogen balance in young dairy calves as affected by antibiotics and age. *J. Animal Sci.* **14**, 1209.

No statistically significant differences were observed in the digestion of dry matter, ether extract, nitrogen-free extract or crude fiber. Chlortetracycline increased the apparent digestion of protein and the nitrogen balance over the control calves. Dry matter, crude fiber, apparent protein digestion and nitrogen balance (per cent of dietary nitrogen retained) for the different groups were:

	Dry matter digestion	Crude fiber digestion	Apparent protein digestion	Nitrogen balance
Control	71.6	48.4	73.3	27.3
Chlortetracycline	72.8	46.8	76.9	35.7
Bacitracin	71.8	47.0	75.2	30.8

268. Hogue, D. E., Warner, R. G., Grippin, C. H. and Loosli, J. K., 1956, Digestion coefficients and nitrogen retention of young dairy calves as affected by antibiotics and advancing age. *J. Animal Sci.* **15**, 788-793.

There was no effect on the digestibility of dry matter, ether extract, crude fiber or nitrogen-free extract. Chlortetracycline increased both the apparent protein digestibility and nitrogen retention ($P = 0.05$). At 16 weeks as compared with 7 weeks, apparent protein digestibility ($P = 0.10$) and nitrogen retention ($P = 0.05$) were also increased.

269. Baumann, C. A., 1956, Test animals. First International Conference, Antibiotics in Agriculture. Nat. Acad. Sci. and Nat. Res. Council, 1956, Washington, pp. 47-54.

Experimental results suggest a more rapid diffusion of xylose through isolated intestines of rats and chicks previously fed antibiotics than is seen in tissue from control animals.

270. Lambert, M. R. and Jacobson, N. L., 1957, The effect of chlortetracycline feeding on *in vivo* cellulose digestion by rumen microorganism. *J. Dairy Sci.* **40**, 633-634.

Nylon bags containing known quantities of cellulose (Solka-Floc) were introduced into the rumen of three fistulated steers and cellulose digestion (as estimated by disappearance from the bags) in a given time was

determined. At 2-week intervals four bags were placed in the rumen of each animal and one was removed after each of the following periods for analysis: 24, 48, 72, and 96 hours. Digestibility values after the above intervals, respectively, were 28.6, 45.3, 59.9, and 64.3% for the controls, and 26.8, 43.2, 52.5 and 64.8% for the antibiotic-fed animals.

271. Horn, L. H., Jr., Snapp, R. R. and Gall, L. S., 1955, The effect of antibiotics upon the digestion of feed nutrients by yearling steers with bacteriological data. *J. Animal Sci.* **14**, 243-248.

Aureomycin and penicillin at different levels of intake were compared in digestion and nitrogen balance trials with steers. The results indicated (1) that aureomycin had a greater depressing effect than penicillin on the digestibility of protein and crude fiber, and (2) that the two supplements had about equal effect in decreasing nitrogen retention.

272. Evans, J. L., Grainger, R. B. and Thompson, C. M., 1955, Effect of various levels and prolonged supplementation of chlortetracycline (Aureomycin) upon roughage digestion by sheep. *J. Animal Sci.* **14**, 1202.

The differences are not statistically significant. The data indicate eventual adjustment of rumen microorganisms to antibiotics supplementation.

273. Thompson, C. M. and Grainger, R. B., 1954, Effect of aureomycin on digestion of low quality roughage by sheep. *J. Animal Sci.* **13**, 1002.

The wethers in Group I received the basal ration; Group II as I, plus 10 mg pure crystalline aureomycin HCl per pound of ration. The wethers in Group II exhibited anorexia within 48 to 72 hours after antibiotic supplementation. Appetites returned to normal within two or three days. The average apparent digestion coefficients for crude fiber were 71.4 and 60.4 for the wethers in Groups I, and II respectively. These differences are statistically significant. There was no difference in nitrogen retention among groups.

274. Huang, T. C. and McCay, C. M., 1953, The effect of terramycin on the growth and body composition of pigs. *J. Nutr.* **50**, 129-139.

An improvement in digestibility of protein was seen in pigs fed the antibiotic.

275. Forbes, R. M., 1954, Studies on the influence of antibiotics and methionine on nitrogen utilization and basal metabolism of the growing male albino rat. *J. Nutr.* **53**, 275-287.

The digestibility of protein nitrogen was greater in animals receiving antibiotic.

276. Thayer, R. H. and Heller, V. G., 1955, Antibiotics and nitrogen utilization in growing cockerels. *Poultry Sci.* **34**, 97-102.

Penicillin and aureomycin increased the amount of nitrogen absorbed from the intestinal tract of the 4-week-old cockerels. An increase in nitrogen retention and decrease in urinary nitrogen excretion were observed in the antibiotic-fed cockerels.

277. Dickison, H. L. and Tisch, D. E., 1954, Effect of procaine penicillin in an amino acid test for rats. *Antibiotics Annual 1954-55*. Proc. Symposium on Antibiotics, Washington, D. C., 516-524.

Procaine penicillin appears to increase the food efficiency when included in an amino acid diet for rats.

Procaine penicillin apparently exerts an amino acid-sparing action.

It is suggested that such a sparing action may be due to enhanced absorption from the intestinal tract.

278. West, J. W. and Hill, J. E., 1955, Protein requirement of broilers as influenced by antibiotics. *Poultry Sci.* **34**, 628-634.

In the presence of the antibiotic, optimal growth and feed efficiency were obtained with the 18% level of protein. When no antibiotic was added to the diet, a protein level of at least 20% was required to produce optimal growth and feed efficiency. These results were interpreted to indicate that the four antibiotics used in this study exerted a "sparing" effect upon the protein requirement of the young chicken.

279. Packett, L. V., Watkins, T. D., Jr. and Kunkel, H. O., 1957, Chlortetracycline in pelleted and unpelleted, urea and cottonseed meal containing rations for fattening lambs with a reference to urinary calculi. *J. Animal Sci.* **16**, 1036.

In the pelleted ration, chlortetracycline did not appear to inhibit the utilization of urea. Urinary calculi were significantly reduced by the inclusion of chlortetracycline in the ration ($P = 0.01$).

280. Richter-Otto, W., 1957, (Nitrogen-balance studies in growing albino rats on supplementing feed with antibiotics) *Ernährungsforschung* **2**, 3-33, (*Chem. Abstr.* **51**, 18174, Nov. 25, 1957.)

Three rats fed a ration containing rye as the protein source during a 10 to 11 day period followed by 10 days during which the ration was supplemented with streptomycin showed no statistical difference in nitrogen retention between the two feeding periods. Among penicillin, streptomycin and aureomycin, in similar tests on young rats, with lactalbumin as the protein source, only streptomycin increased nitrogen retention. It is concluded that the protein-sparing action of antibiotics with swine cannot be explained on the basis of increased nitrogen retention.

281. Wacker, A., Heyl, W., Buechl, H. and Holthoff, H. J., 1956, The action of antibiotics as growth substances in animals. II. Studies with inactivated penicillin and copper sulfate. *Arzneimittel-Forsch.* **6**, 712-714, 1956. (*Chem. Abstr.* **51**, 5938, April 25, 1957.)

Addition of 40 mg cysteine, taurine, or inactivated penicillin per kg diet containing 2% protein results in improved growth of baby chicks. Penicillin is the most effective compound. A combination of penicillin and CuSO_4 added to the diet has the same growth-promoting effect as a diet containing 12% protein.

282. Ferrando, R., Bost, J. and Brenot, D., 1953, Action des antibiotiques sur l'absorption intestinale (Action of antibiotics on intestinal absorption). *Compt. rend. Acad. d. Sc.* **236**, 1618-1620. (*Chem. Abstr.* **47**, 7656, Aug. 10, 1953.)

Casein hydrolyzate was introduced into an isolated portion of ileum in situ in the rat under urethan anesthesia. The absorption of N/unit time was increased in about 80% of the trials where 1.2 mg of procaine penicillin and 1.2 mg of aureomycin HCl had been added per milliliter of hydrolyzate. It is suggested that the antibiotics altered intestinal permeability.

283. Draper, H. H., 1958, The absorption of radiolysine by the chick as affected by penicillin administration. *J. Nutr.* **64**, 33-42.

Absorption of C^{14} L-lysine was faster in chicks fed antibiotic. These chicks had lighter intestinal tract than controls.

284. Chance, C. M., Duncan, C. W., Huffman, C. F. and Luecke, R. W., 1953, Antibiotics in rumen digestion and synthesis. II. The effect of Aureomycin on the concentration of some amino acids and B vitamins in the rumen. *J. Dairy Sci.* **36**, 495-503.

The concentration of the 10 essential amino acids in the rumen six hours after feeding was less when 0.5 gm of aureomycin was included in the ration. The data suggest that the rate of removal from the rumen was accelerated. Synthesis of nicotinic acid was indicated during the first 12 hours after feeding the aureomycin-free ration, whereas both levels of aureomycin tended to reduce the amount of synthesis.

285. Carroll, R. W., Hensley, G. W., Sittler, C. L., Wilcox, E. L. and Graham W. R., Jr., 1953, Absorption of nitrogen and amino acids from soy-bean meal as affected by heat treatment or supplementation with aureomycin and methionine. *Arch. Biochem. and Biophys.* **45**, 260-269.

The addition of aureomycin to either raw or heated meal resulted in improved absorption of each of the four amino acids during passage of the ration through the small intestine.

286. Hartsook, E. W. and Johnson, B. C., 1953, Effects of dietary terramycin and methionine supplements on fat and protein gains in weanling rats. *J. Nutr.* **51**, 205-218.

The terramycin-methionine interaction: (a) increased carcass N deposition non-significantly (4.5%, $P = 0.2$) and increased the efficiency of N utilization significantly (10.7%, $P = 0.01$): (b) significantly decreased carcass ether extract deposition (30.8%, $P = 0.05$) and significantly decreased the efficiency of utilization of dry matter for carcass ether extract deposition (30.6%, $P = 0.05$). Thus one may infer that the terramycin-methionine interaction enhanced the efficiency of utilization of dietary N and depressed fat gains.

287. Waisman, H. A. and Boldt, L. C., 1957, Influence of antibiotics and tryptophan deficiency on growth in the rat. *J. Nutr.* **61**, 457-463.

Terramycin, achromycin and erythromycin at 0.01% gave the same growth response as 0.2% tryptophan in rats fed 9% casein. The effect was not seen in female rats.

288. Braham, J. E., Bird, H. R. and Baumann, C. A., 1957, Effect of antibiotics on folic acid deficient chicks. *Federation Proc.* **16**, 382.

On a practical ration low in folic acid, procaine penicillin produced a 28% average increase in growth and chlortetracycline 16%. In another series with a diet containing 0.25 mg of folic acid/kg responses to procaine penicillin and chlortetracycline were 8 and 2%, respectively, but responses to streptomycin, chloromycetin and bacitracin at levels of 35 ppm were, respectively, 49, 35, and 16%. Analyses of livers showed that the effects of procaine penicillin and chlortetracycline upon folic acid storage were roughly comparable to effects upon growth.

289. Libby, D., 1952, Effect of dietary penicillin on some B-vitamin requirements in the chick. Thesis, Michigan State College.

Penicillin was found to exert a sparing action on calcium pantothenate (levels of pantothenate ranged from 0.2 mg% to 1.0 mg%). This effect was especially evident in the group receiving the 0.4 mg% level of panto-

thenic acid. Feed efficiencies were improved by penicillin in those groups receiving the lower levels of pantothenate.

290. Briggs, G. M., Spivey, M. R. and Ortiz, L. O., 1953, Growth depressing effect of Aureomycin in folic-acid deficient chicks. *Federation Proc.* 12: 410.

Deficient chicks weighed an average of 231 gm at 4 weeks (8 experiments, 6 chicks/group as compared with 261 gm for controls fed pteroyl-glutamic acid (2 mg/kg of diet). Crystalline aureomycin, at levels of 20 to 500 mg/kg in the folic acid-deficient diet, further reduced the body weight to an average for about 130 gm. Levels below 20 mg had no consistent influence on growth. Terramycin, chloromycetin and sulfasuccidine produced growth retardation in the folic acid-deficient chicks, whereas, procaine penicillin G and bacitracin had no influence.

291. Nelson, T. S. and Scott, H. M., 1953, Niacin deficiency in the chick as influenced by antibiotics. *Poultry Sci.* 32, 601-604.

Niether aureomycin nor penicillin stimulated chick growth when the diet was severely low in niacin (0.48 mg/kg). In contrast, both antibiotics significantly increased chick growth when the diets contained adequate or slightly suboptimal levels of the vitamin. This was especially true for the chicks fed the sucrose basal.

292. Daft, F. S. and Schwarz, K., 1952, Prevention of certain B vitamin deficiencies with ascorbic acid or antibiotics. *Fed. Proc.* 11, 200-201.

Rats fed a riboflavin deficient diet survived for one year if 200 ppm chlortetracycline was added to the diet.

293. Dickison, H. L. and Tisch, D. E., 1955, Effect of procaine penicillin in an amino acid diet for rats. *Antibiotics Annual 1954-55*, pp. 516-524.

When rats were fed diets with amino acids as the sole protein source, procaine penicillin allowed life to continue for 26 weeks with no B-vitamins in the diet. Growth stopped at about 11 weeks. When either adequate or low levels of the amino acid mixture were fed (in the presence of B-vitamins) the growth was improved in the presence of procaine penicillin.

294. Waisman, H. A., Green, M., Munoz, J. C., Ramenchik, A. and Richmond J. B., 1951, Role of Aureomycin and Citrovorium factor in "folic acid" deficiencies. *Proc. Soc. Exptl. Biol. and Med.* 76, 384-388.

Oral or injected Aureomycin can counteract the effect of aminopterin, and also stimulate growth on a diet with no folic acid added.

295. Cravioto-Munoy, J., Poncher, H. G. and Waisman, H. A. 1951, Vitamin B₁₂ sparing action of aureomycin in the rat. *Proc. Soc. Exptl. Biol. and Med.* 77, 18-19.

Aureomycin can replace vitamin B₁₂ in the diet for a rat.

296. Nickell, L. G., 1955, Effects of antigrowth substances in normal and atypical plant growth. Pp. 129-151, in C. P. Rhoads, "Antimetabolites and Cancer." A.A.A.S., Washington.

Antihormones are substances as malic hydrazide which inhibit plant growth at extremely low levels without showing any stimulation. Antibiotics has shown inhibitory results in plants because other workers never used levels below 50 ppm. At concentrations of 1-5 ppm, penicillin G, oxytetracycline, streptomycin, bacitracin or thiolutin replaced the thiamin requirement of *Rumex* virus tumor tissue for several trans-

fers. Antibiotics also increased germination amount and growth rates of several plants in sterile and nonsterile soil. Any of the same antibiotics stimulated growth of *Lemna minor* in aseptic culture. The possibility that these were acting as chelating agents was strengthened by the growth stimulation obtained with EDTA. Several proposals for the mechanisms of action are listed.

297. Giunchi, G., Fidanza, A. F., Seuro, L. A. and Sorice, F., 1954, The influence of some antibiotics on growth and the production of antibodies in rats fed a pantothenic acid deficient diet. *Exptl. Med. Surg.* **12**, 430-433.

Antibiotics increased the growth and ability of pantothenic acid deficient rats to produce antibodies.

298. McKigney, J. I., Wallace, H. D. and Cunha, T. J., 1957, The influence of chlortetracycline on the requirement of the young pig for dietary pantothenic acid. *J. Animal Sci.* **16**, 35-43.

Five-week old weanling pigs in drylot developed pantothenic acid deficiency symptoms on a 14% crude protein basal ration composed of ground corn, soybean oil, meal, minerals and supplemented with vitamins except for pantothenic acid. Supplementation of this ration with either pantothenic acid or chlortetracycline prevented the development of deficiency symptoms.

299. Olivares, E. and Masankay-Arenas, L., 1954, The effect of CME (cow manure extract) as feed supplement on growing pigs as compared to some antibiotic feed supplement. *Phillippine J. Animal Industry* **15**, 237-245. (*Biol. Abstr.* **30**, 34878, Dec. 1956.)

In feeding trials with pigs cow manure extract proved to be equal to antibiotic (Aurofac), when added to a standard ration.

300. Halevy, S., Diamant, E. J. and Guggenheim, K., 1955, The effect of antibiotics on the metabolism of nicotinic acid, biotin and folic acid in rats. *British J. Nutrition* **9**, 57-62.

Under their experimental conditions, aureomycin seemed to exert a weak sparing action on nicotinic acid, folic acid and biotin, and oxytetracycline on nicotinic acid and folic acid. In contrast, streptomycin appeared to increase the requirements of rats for biotin and folic acid.

301. McDaniel, E. G. and Daft, F. S., 1955, Effects of penicillin and aureomycin on rats fed pyridoxine-deficient diets. *Federation Proc.* **14**, 443.

Weanling rats were fed purified diets containing 18% casein, 8% fat, 4% salts, dextrose and vitamins. Twelve rats/group were used and comparisons were made between litter mates. Omission of pyridoxine from the otherwise adequate diet resulted in reduced growth and survival and in the development of dermatitis. Inclusions of 100 or 200 mg% procaine penicillin G in the deficient diet resulted in a marked beneficial effect on growth and on survival and the incidence and severity of the dermatitis was diminished. The inclusion of 20 or 40% mg of aureomycin in the deficient diet had a marked but opposite effect, growth was greatly diminished (as compared to the deficient controls), while the incidence and severity of the dermatitis and the mortality were increased. Penicillin was not effective in preventing the deleterious effects of aureomycin; animals which received both antibiotics in the deficient diet behaved very similarly to those receiving aureomycin alone.

302. Murray, T. K. and Campbell, J. A., 1955, The effect of aureomycin on the apparent utilization of vitamin A by the ovariectomized rat. *J. Nutr.* **57**, 89-99.

Aureomycin increased significantly the response to vitamin A. This effect was relatively transient. Experiments in which aureomycin supplementation was begun 24 hours after the last vitamin A dose suggested that the effect was not a result of increased absorption of the dose. The addition of vitamin B₁₂ to the diet did not mask the effect of Aureomycin on the vitamin A assay. Likewise the addition of tocopherol to the vitamin A doses had no influence on the vitamin A assay in either the presence or absence of aureomycin.

303. Jones, J. D. and Baumann, C. A., 1955, Relative effectiveness of antibiotics in rats given limiting B vitamins by mouth or by injection. *J. Nutr.* **57**, 61-71.

Limiting amounts of thiamine, riboflavin and pantothenic acid were administered to rats either by inclusion in the diet or by subcutaneous injection, and the effectiveness of dietary antibiotics on growth determined. Penicillin and Aureomycin consistently increased growth under both conditions, but the magnitude of the increase was usually greater when the limiting B vitamin was in the diet than when it was injected. This was true in five or six experiments with thiamine, six of six with pantothenic acid, and three of five with riboflavin.

304. Murray, T. K. and Campbell, J. A., 1955, Effect of aureomycin on liver storage of vitamin A, and on growth, depletion and survival time of rat. *J. Nutr.* **57**, 101-110.

Aureomycin makes available amounts of vitamin A which affect survival time and depletion time, but which are not large enough to influence significantly the liver stores.

305. Hartsook, E. W., 1956, A study of the effect of chlortetracycline (aureomycin) upon calcium retention by the growing male albino rat. *J. Nutr.* **60**, 97-104.

Aureomycin supplementation of the basal diet did not increase body weight gain, carcass water gain, body length, carcass calcium gain or the percentage of calcium retention. Aureomycin did, however, increase (10.7%) the carcass ether extract gain to an extent that closely approached statistical significance at the 5% level and significantly decreased (3.5%) carcass dry matter gain.

306. Jenkins, K. J., Bell, J. M., O'Neill, J. B. and Spinks, J. W. T., 1954, The effects of antibiotics on the synthesis of vitamin B₁₂ in the chick. *Can. J. Biochem. Physiol.* **32**, 628-635. (*Nutrition Abstr. and Rev.* **25**, 2020, April 1955.)

A diet lacking vitamin B₁₂, with or without addition of radioactive cobalt as 60 CoCO₃, was given to chicks having penicillin, streptomycin, Aureomycin, Terramycin or no supplement. Cobalt alone did not affect weight gains, but gains were increased when cobalt and an antibiotic were given. The excretion of cobalt rose and that of vitamin B₁₂ fell in chicks receiving antibiotics. Estimation of cobalt and vitamin B₁₂ at all levels of the gastrointestinal tract and in tissues showed that synthesis of vitamin B₁₂ began in the proventriculus and that the rate and site of synthesis were affected by the antibiotics. The amount of

cobalt in the tissues was higher when no antibiotic was given. The vitamin B₁₂ content of all tissues, except the blood and gallbladder which showed high values, was little affected by administration of antibiotics. It is suggested that in chicks given no antibiotic the high values found in the blood and gall-bladder were a sign of defective utilization of the vitamin.

307. Carrick, C. W., Rogler, J. C. and Hauge, S. M., 1954, Discover riboflavin needs for chicks up to 6 weeks. 67th Ann. Rept. Agric. Exper. Sta. Purdue Univ., p. 49.

A factorial experiment with three levels each of riboflavin and the antibiotic aureomycin failed to show that the use of the antibiotic lowered the requirement for riboflavin, but rather indicated that the antibiotic tended to increase the requirements. A statistical analysis of the data showed a significant interaction between sex and the amount of aureomycin included in the ration. The male chicks required a higher level of aureomycin for maximum growth than the females.

308. Machlin, L. J., Denton, C. A., Kellog, W. L. and Bird, H. R., 1952, Effect of dietary antibiotic upon feed efficiency and protein requirements of growing chickens. *Poultry Sci.* **31**, 106-109.

The protein requirement for early growth of chickens appeared to be decreased slightly by addition of aureomycin to the diet. Aureomycin stimulated growth most effectively when added to a diet containing 19% protein. It was less effective with lower levels. Aureomycin increased efficiency of feed utilization when added to a corn-soybean diet containing vitamin B₁₂. The effect of the aureomycin was more pronounced with increasing protein levels.

309. Asenjo, C. F. and Pomales-Lebron, A., 1952, Effect of large oral dosages of aureomycin on the splenic lesions developed by rats depleted of folacin. *Federation Proc.* **11**, 435.

It can be concluded, however, that under the conditions of the experiment, aureomycin does not prevent the formation of splenic lesions in folacin depleted rats.

310. Sauberlich, H. E., 1952, Effect of aureomycin and penicillin upon the vitamin requirements of the rat. *J. Nutrition* **46**, 99-107.

The addition of 0.01% penicillin to a casein sucrose synthetic basal ration caused a marked growth stimulation in weanling rats fed diets free of, or low in, thiamine, pyridoxine, pantothenic acid and, to some extent, riboflavin. Aureomycin at the same level had a similar effect in the case of pantothenic acid and thiamine deficiencies. The effect of the antibiotics on thiamine and pyridoxine deficiencies was evident whether sucrose or dextrin was used in the diet. The addition of penicillin or aureomycin in the completely vitamin supplemented basal ration had no effect upon the growth of the animals.

311. Fidanza, A., Guinchi, G., Rutigliano, M. L., Scuro, L. A. and Sorice, F., 1953, Influenza di vari antibiotici sull'accrescimento di giovani ratti alimentati con dieta sintetica, priva di acido pantothenico (Action of antibiotics on the growth of young rats fed a synthetic diet deficient in pantothenic acid). *Bull. Soc. Ital. Biol. Sper.* **29**, 69-71. (*Chem. Abstr.* **48**, 825, Jan. 25, 1954.)

312. Guggenheim, K., Halevy, S., Hartmann, I. and Zamir, R., 1953, The effect of antibiotics on the metabolism of certain B vitamin. *J. Nutr.* **50**, 245-253.

The addition of these antibiotics to the diet (50 mg/kg) caused a marked stimulation of the growth of rats fed diets low in riboflavin and pantothenic acid. A similar effect was observed with penicillin and Terramycin added to a low thiamine diet, but not with Aureomycin and Streptomycin.

313. McKigney, J. I., Wallace, H. D. and Cunha, T. J., 1957, The influence of chlortetracycline on the requirement of the young pig for dietary pantothenic acid. *J. An. Sci.* **16**, 35-43.

At 10 mg/lb chlortetracycline had a sparing effect on the dietary requirement of pigs for pantothenic acid.

314. Lih, H. and Bauman, C. A., 1951, Effect of certain antibiotics on the growth of rats fed diets limiting in thiamine, riboflavin or pantothenic acid. *J. Nutrition* **45**, 143-151.

Penicillin, streptomycin, and aureomycin stimulated the growth of rats receiving limiting amounts of thiamine, penicillin being the most effective.

315. Schendel, H. E., 1952-54, Studies on the mechanism of action of antibiotics in stimulating growth of animals on marginal or deficient diets. *Res. Prog.* **11**, *Agric. Exper. Sta. Rept.*, pp. 54-55.

Radiothiamin distribution in tissues and excreta demonstrated penicillin spares thiamin by increasing its intestinal synthesis.

316. Common, R. H., Keefe, T. J., Burgess, R. and Maw, W. A., 1950, Modification of the biochemical response in the immature pullet to estrogen by means of dietary aureomycin. *Nature* **166**, 992-993.

Calcium absorption is increased by dietary antibiotics.

317. Bartley, E. E., Fountaine, F. C. and Atkeson, F. W., 1953, Antibiotics in dairy cattle nutrition. II. Effects of feeding on aureomycin product (Aureofac 2A) to lactating dairy cows. *J. Dairy Sci.* **36**, 402-408.

When Aureofac-2A was fed, the cows produced on an average 0.15 lb more fat corrected milk daily, consumed from 0.2 to 0.4 lb more alfalfa hay daily and 0.15 lb less water daily than when no Aureofac-2A was fed. None of these differences were statistically significant. Body weight, rumination, grain and silage consumption, pulse rate, body temperature and general health and well being also were unaffected by Aureofac-2A feeding.

318. Rusoff, L. L., Haq, M. O., Branton, C., Patrick, T. E. and D'Arensbourg, G., 1952, Report on feeding aureomycin supplement to mature dairy bulls and lactating dairy cows. *J. Animal Sci.* **11**, 774-775.

The semen characteristics and fertility data were as follows: for the control group—no. of ejaculates, 98, and for the aureomycin group (300 mg/day), 110; vol. per ejac. (ml) 5.50 and 5.33; sperm per ml $\times 10^6$, 1,514 and 1,485; sperm per ejac. $\times 10^6$, 8,042 and 8,456; per cent initial motility, 50.4 and 46.6; motility sperm per ejac. $\times 10^6$, 4,164 and 3,918; first services 979 and 1,141; non returns 627 and 773; and per cent of 60-90 showed no significant differences between treatments. Two comparable groups of 5 lactating Holstein cows each were fed a 15.5% digestible protein grain ration. One group served as a control and the

other received an aureomycin supplement (1% level) so that approx. 130 mg of aureomycin was consumed daily per animal over a period of 60 days. Milk production records for both groups showed no significant difference in the amount of 4% fat-corrected milk. The composition of the milk for butterfat, total solids, solids-not-fat, protein, titratable acidity, pH, ash, Ca and P was very similar for both groups. The bacterial count of the milks also showed no significant difference between the groups. No evidence of any physiological effect was observed in the feeding of aureomycin to the cows or bulls.

319. Jacobs, R. L., Elam, J. F., Tidwell, W. L., Flower, J. and Couch, J. R., 1954, Effect of antibiotics on reproductive performance and antibiotic resistant organisms in the digestive tract of the hen. *Antibiotics Annual 1954-55*. Proc. Symposium on Antibiotics, Washington D. C., Oct., pp. 525-529.

The addition of high and low levels of oxytetracycline or bacitracin to a diet complete with respect to vitamins and unidentified factors increased egg production and fertility during a six month test period. A high level of chlortetracycline resulted in an increase in egg production. Antibiotic supplementation had no effect on hatchability of eggs from hens fed a complete diet.

320. Carlson, C. W., Wilcox, R. A., Kohlmeyer, W. and Jones, D. G., 1955, Reproductive performance of chickens as influenced by antibiotics in the diet. *South Dakota Agric. Exper. Sta. Tech. Bull.* 15.

Where egg production was improved, feed efficiency was likewise improved. Body weight maintenance, mortality, egg quality and hatchability of fertile eggs were not consistently affected by the antibiotics. Progeny growth appeared to be somewhat retarded in a few instances, although as a whole, where improved chick starter diets were used, there were no consistent effects. There were several instances in which it appeared that progeny growth was improved by antibiotics in the breeder diet.

321. Elam, J. F., Jacobs, R. L. and Couch, J. R., 1953, The effect of prolonged feeding of antibiotics upon the performance of laying hens. *Poultry Sci.* 32, 792-795.

Prolonged feeding of antibiotics resulted in increased egg production and hatchability. Parenteral administration of these antibiotics in water failed to increase the hatchability of eggs and to decrease the number of eggs exhibiting typical vitamin B₁₂ deficiency symptoms observed in the embryos. Parenteral administration of inactivated penicillin in water and penicillin in oil increased egg production and hatchability.

322. Halick, J. V. and Couch, J. R., 1951, Antibiotics in mature fowl nutrition. *Pjoc. Soc. Exper. Biol. and Med.* 76, 58-62.

Streptomycin appeared to maintain a somewhat higher level of hatchability during the second through the fourth week than did the unsupplemented diet or vitamin B₁₂ injections but during the fifth through the eighth week the level declined more than in the hens receiving vitamin B₁₂. The feeding of aureomycin or penicillin alone apparently assisted in the depletion of the birds of vitamin B₁₂ and possibly of

the unidentified factor, and the feeding of these antibiotics with vitamin B₁₂ was less effective than B₁₂ alone.

323. Carlson, C. W., Wilcox, R. A., Kohlmeyer, W. and Jones, D. G., 1953, The effect of penicillin and streptomycin in diets for breeding hens. *Poultry Sci.* **32**, 176-178.

In three of the four experiments, penicillin slightly improved egg production, whereas streptomycin did so in all cases. Vitamin B₁₂ had little effect on the egg production of the control hens, whereas the production of hens given B₁₂ and antibiotics was greater than in those given antibiotics alone. The hens receiving penicillin or streptomycin supplements showed a greater per cent hatchability of fertile eggs than the hens on the control diets. There was an indication that the antibiotic-fed hens produced slightly smaller eggs.

324. Price, J. D., Stelzner, H. D., Reid, B. L. and Couch, J. R., 1956, Effect of antibiotics and arsonic acids on egg production. *Poultry Sci.* **35**, 1165-1166.

Egg production was increased 10% by feeding 25 mg per pound aureomycin and was not further increased by feeding 50, 150 and 200 mg of the antibiotic per pound. The feeding of 3-nitro-4-hydroxy phenylarsonic acid, 22.5 mg per pound, increased egg production 4 to 6% and improved feed efficiency.

325. Carlson, C. W., 1957, Some effects of arsanilic acid and/or penicillin upon egg production. *Poultry Sci.* **36**, 1070-1075.

Arsanilic acid (at 90 or 120 gm/ton) addition to breeder diets improved egg production from 1.8% to 4.2% over a 7 or 9 month period with a corresponding reduction in feed requirements of from 0. to 0.5 lb per dozen eggs produced.

Hatchability of all eggs set was improved by arsanilic acid. There was little effect upon egg size, Haugh unit value, egg shell thickness or progeny growth.

Arsanilic acid or penicillin fed during the growing period did not appreciably affect the time required for pullets to attain 50% production.

326. Sherwood, D. H. and Milby, T. T., 1953, Antibiotics in the ration of laying and breeding hens. *Poultry Sci.* **32**, 932.

Several experiments have been conducted to test the effect of adding antibiotic to the ration of laying and breeding hens. These tests include caged layers, as well as birds housed in the conventional manner. No effect was seen.

327. Bearse, G. E. and Berg, L. R., 1955, Adding antibiotics to diets of good layers not necessary. *Flour and Feed* **56**, 4-5.

A small significant change to a slightly darker yolk color was brought about by the addition of aureomycin to the feed. Hatchability was not influenced by any of the treatments.

328. Sizemore, J. R., Lillie, R. J., Denton, C. A. and Bird, H. R., 1953, Influence of aureomycin in the chick diet upon subsequent reproductive performance of laying hens. *Poultry Sci.* **32**, 618-624.

Feeding antibiotic (5, 10, 20 and 40 mg Aureomycin/kg diet) to growing pullets had no effect on mortality or date of sexual maturity.

There was no influence of chick diet on mature body weight, laying house mortality, egg production, fertility, egg weights or egg shell

thickness. Among pullets fed a vitamin B₁₂ deficient breeder diet there was a profound influence of chick diet upon the hatchability for fertile eggs.

329. Sherwood, H. D. and Milby, T. T., 1954, Further tests with antibiotics for laying and breeding hens. *Poultry Sci.* **33**, 1031-1033.

Experiments with antibiotics (oxytetracycline-chlortetracycline mixture, 100 mg/lb) involving over 1,500 laying birds fed practical type rations have been reported. There was more variation between hens than between treatments, but results failed to indicate any improvement in egg production, feed efficiency or hatchability when antibiotics were included in the ration. Mortality was similar in the control and in the antibiotic group.

330. Peterson, C. F. and Lampman, C. E., 1952, Value of antibiotics in rations for egg production. *Poultry Sci.* **31**, 1067-1069.

The addition of approximately 9 gm of either procaine penicillin, streptomycin, or terramycin hydrochloride per ton of an animal-vegetable protein laying mash for White Leghorn pullets during the first year of egg production did not improve egg production, livability or body weight and did not reduce losses from specific diseases such as avian leukosis and blue comb.

331. Slinger, S. J., Morphet, A. M., Hunt, E. C. and Pepper, W. F., 1953, Effect of penicillin on the reproductive performance of turkeys. *Poultry Sci.* **32**, 660-669.

The addition of penicillin (2 gm/ton feed) to a practical turkey breeder diet resulted in a decrease in hatchability, egg production, egg weight and feed consumption.

332. Heywang, B. W., 1957, The relative effect of two high levels of an antibiotic on laying chickens during hot weather. *Poultry Sci.* **36**, 871-873.

Chlortetracycline at the levels of 0, 50, and 100 gm, respectively, per ton of diet was fed to groups of 30 laying White Leghorns during three periods of 112 days each. Each diet was fed to the same two groups during only one of the 112 days periods. A statistical analysis of the data showed a significantly greater egg production in the groups fed the supplement than on the other groups during hot weather only. There was little difference in the egg production of groups fed the two levels of supplement.

The quantity of diet consumed per dozen eggs laid was lower during hot weather only when the supplement was in the diet, and there was little difference in the effect of the two levels.

333. Carlson, C. W., Wilcox, R. A. and Kohlmeyer, W., 1955, Antibiotics in the maternal diet as affecting growth of the progeny. *Poultry Sci.* **34**, 1187.

A consideration of the growth response to antibiotics and to other recognized growth promotants showed few consistent differences between control progeny and progeny from antibiotic-fed hens. In general, it appears that there need be little concern over possible detrimental effects of antibiotics in the breeder diet upon growth of the progeny.

334. Slinger, S. J., Morphet, A. M., Hunt, E. C. and Pepper, W. F., 1954, Effect of penicillin and forage juice on reproduction and growth of turkeys. *Poultry Sci.* **33**, 944-951.

The rate of growth of poults hatched from hens fed forage juice was not different to that of poults hatched from hens not receiving the sup-

plement. Poults hatched from hens fed penicillin grew more rapidly than those from hens not receiving the antibiotic only when the poult diet contained penicillin and forage juice.

335. Slinger, S. J., Pepper, W. F., Morphet, A. M. and Evans, E. V., 1953, Effect of penicillin on the niacin requirement of turkeys and a carry-over effect of penicillin from dams to progeny. *Poultry Sci.* **32**, 754-762.

Turkeys hatched from hens receiving penicillin weighed less at 8 weeks of age than those hatched from hens not fed the antibiotic. This influence was still apparent in groups fed certain diets at 24 weeks of age.

336. Ryan, F. A., Singen, E. P., Matterson, L. D. and Potter, L. M., 1957, The continuous feeding of an antibiotic to laying hens. *Poultry Sci.* **36**, 1154.

Results may be summarized as follows: (1) egg production, on a hen-day basis, averaged 68.34% for the controls and 72.00% for the treated birds. The difference, 3.66%, is statistically highly significant. (2) There was no significant difference in mortality due to treatment. (3) The egg production differences in favor of the treated group, at the end of 3, 8, 12, 16 and 20 weeks of the experiment, were 3.63, 4.63, 5.35, 5.75 and 7.68% respectively. After 20 weeks, the difference declined steadily. (4) The feed requirement per dozen eggs was 5.16 lb for the treated group and 5.45 lb for the controls. (5) Variation in response within breeds was as great as the variation between breeds.

337. Richardson, L. R. and Russell, E. L., 1951, Folic acid, crystalline vitamin B₁₂ and penicillin in reproduction in rats. *Fed. Proc.* **10**, 391.

Addition of penicillin to the ration of female rats during growth and reproduction did not affect their performance when the diet was balanced nutritionally.

338. Uram, J. A., French, C. E., Barron, G. P. and Swift, R. W., 1954, Terramycin or streptomycin in growth, reproduction and lactation. *Federation Proc.* **13**, 481.

The animals were bred at 4 months without significant differences in reproductive ability. Weight gain of young of animals on Terramycin supplement during the 3rd to 14th days, post partum (lactation performance), was significantly increased over control diet young. Dams were rebred one month after weaning. This second reproduction of dams on all diets was considerably diminished by unknown causes, presumably age. Dams on Terramycin had the best reproduction.

339. Uram, J. A., French, C. E., Barron, G. P. and Swift, R. W., 1955, The effect of high levels of terramycin or streptomycin on growth, reproduction and lactation of the rats. *J. Nutr.* **55**, 481-492.

340. Wallace, H. D., 1955, A long time study on the feeding of chlortetracycline (Aureomycin) to gestating-lactating sows. *J. Animal Sci.* **14**, 1225.

A study has been completed to determine the long time effect of adding chlortetracycline at the rate of 40 gm per ton to the ration of gestating-lactating sows fed on good grass and legume pastures. First-, second-, third-, fourth- and fifth-litter performances indicate that the antibiotic supplemented sows have farrowed slightly heavier pigs, weaned slightly fewer pigs and weaned less total weight per litter. There is no clear evidence from the experiment that the antibiotic has been either detrimental or beneficial over this long-time feeding period.

341. White-Stevens, R. H., 1957, Antibiotics as dietary supplements for poultry. *The Vet. Rec.* **69**, (8-11) 217-229.

Dietary antibiotics are essential for concentrated broiler production areas which are found in certain areas of the United States.

342. Whitehair, C. K., 1952, *Vet. Sci. News.* (University of Wisconsin), p. 6.

343. Coates, M. E. and Kon, S. K., 1955, Bacteriological implication of growth stimulation by antibiotics in different environments. *Proc. 3rd International Congress of Biochemistry*, pp. 448-52. Academic Press, N. Y., 1955.

The environmental factor in the growth response obtained in chicks fed antibiotics. She would suggest "dirty" (or used) chick quarters contain an infectious agent, which can be found in the gut contents, which may lead to poor growth via a "sub-clinical infection."

344. Cooper, D. M. and Gordon, R. F., 1955, Possible mode of action of antibiotics in chicks. *J. Sci. Food Agr.* 1-154.

345. Gouge, H. E., Elliott, R. F. and Van Roekel, O. K., 1958, Effect of chlortetracycline on incidence of cervical abscesses and weight gains of swine. *J. Animal Sci.* **17**, 34-41.

Chlortetracycline (50 or 100 mg per ton) reduced cervical abscesses and weight gains were increased, feed efficiency was improved.

346. Richardson, D., 1955, Antibiotics for growing-fattening swine. Circular 320, Kansas State College, p. 34.

The average daily gain of pigs fed the unsupplemented diet rose from 1952 to 1954 while the response to dietary antibiotics decreased.

347. Dubos, R. J., 1956, Retrospectives and prospectives. *Proc. 1st International Con. on the Use of Antibiotics in Agriculture.* Nat. Acad. Sci. and Nat. Res. Council, Washington, pp. XV-XX.

Professor Pangloss should have been less dogmatic in selling the world to Candide.

348. Radisson, J. J., Bartley, E. E., Lord, T. H. and Swenson, M. J., 1956, The mode of action of antibiotics in the nutrition of the dairy calf. II. Effect of Aureomycin administered orally to young dairy calves on the sensitivity of intestinal bacteria to phagocytosis. *J. Dairy Sci.* **39**, 1386-1395.

Oral administration of aureomycin (45 mg/calf/day and 125 mg/calf/day) did not materially affect phagocytic activity of the leucocytes in the blood of the calves. However, bacteria isolated from feces of calves receiving aureomycin were more sensitive to phagocytosis than bacteria isolated from feces of the calves receiving no aureomycin. This difference was significant statistically. Phagocytic sensitivity was further increased when bacteria were grown *in vitro* in the presence of sub-bacteriostatic concentrations of aureomycin. Leucocytes from young calves (birth to 2 weeks) had a lower phagocytic activity than leucocytes from older calves (5 to 7 weeks). It is possible, therefore, that antibiotics may play a greater part in the defense of the body and thereby promote growth to a greater extent during early life when body defenses are inadequate, and when incidence of intestinal disturbances is greater and growth rate is lower than in later life.

349. Klosterman, E. W., Moxon, A. L. and Cahill, V. R., 1956, Relative value of the subcutaneous implantation of stilbestrol, feeding stilbestrol and

feeding a combination of stilbestrol and Terramycin, 1956. Rept. Beef Cattle Res., Ohio Agric. Exper. Sta.

There was no advantage of feeding the antibiotic in addition to stilbestrol.

350. Dyer, I. A., Ensminger, M. E. and Blue, R. L., 1957, Effects of fat, oxytetracycline and stilbestrol on performance and hepatic stores of carotene and vitamin A. *J. Animal Sci.* **16**, 828-832.

Oxytetracycline fed steers grew faster and had higher liver vitamin A content than control steers. The hormone had no additive effect on growth or vitamin A content of the liver.

351. Braude, R., Campbell, R. C., Lucas, I. A. M., Luscombe, J. R., Robinson, K. L. and Taylor, J. H., 1955, Antibiotics and endocrine stimulants as promoters of growth in fattening pigs. *British J. Nutrition* **9**, 191-196.

Marked variation in response to a given treatment was recorded at the different centers, probably because of the use of different sources of pigs and the prevalence of virus pneumonia in most of the centers. At one center, toxicity symptoms were observed, apparently associated with the feeding of stilbestrol. In two out of three centers, a significant improvement in the rate of growth was obtained by adding L-thyroxine to a diet containing an antibiotic, over and above the improvement obtained with the antibiotic alone.

352. Lucas, I. A. M. and Calder, A. F. C., 1955, Thyroxine, stilbestrol and antibiotics in rations for castrated male pigs. *British J. Nutrition* **9**, 267-279.

There was no significant overall effect on growth rate from weaning to slaughter with both hormones in diets containing Aurofac or penicillin. However, supplementing the diet containing penicillin with both hormones significantly improved efficiency of food conversion by 5%, but supplementing the diet containing Aurofac with both hormones significantly lowered efficiency of food conversion by 5%.

There were no significant treatment effects upon killing out percentage or upon the majority of carcass measurements, but pigs fed the diet containing hormones and penicillin had the least fat over the "eye" muscle as measured from bacon rashers cut at the level of the last rib.

353. Turner, A. W. and Hodgetts, V. E., 1952, Depression of ruminal digestion in adult sheep by aureomycin. *Australian J. Agric. Res.* **3**, 453-459.

In 3 adult sheep which received a single large dose of aureomycin HCl (23.6 to 27.1 mg/g body wt.), the ruminal flora decreased about 75% in two hours and remained at the low level for at least two days. Ruminal fermentation was greatly depressed. The yield of organic acids reached only about 30% of normal and was insufficient to lower the ruminal pH. Appetite and body weight decreased, but responded favorably to administration of ruminal fluid and yeast.

354. Quin, A. H., 1952, Newer problems in swine diseases—control and treatment. *Canad. J. Comp. Med.* **16**, 265-270.

There is little argument but that pig feeds fortified with antibiotic residues and vitamin B₁₂ will step up growth and bring added profits. However, this past year, several instances of trouble following excessive or prolonged intake of antibiotic residues have been reported. The clinical picture varies, but some droves develop persistent scouring,

encephalitis symptoms, excessive thirst and a variable death loss. Upon autopsy, these pigs show pigmented patches of various color throughout the alimentary tract and in one drove, the entire intestinal mucosa was hidden by a gray-colored mold growth. In another, the esophageal-gastric junction was occluded by a piled up inflammatory fungus lesion which yielded a pure culture of monilia. Evidence that antibiotic residues are related to causation is proved by the fact that further trouble and losses stop when antibiotic intake is eliminated in herds of this type.

355. Bridges, J. H., Dyer, I. A. and Burkhard, W. C., 1952, Effects of penicillin and streptomycin on the growth rate and bacterial count in the feces of pigs. *J. Animal Sci.* 11, 474-479.

Pigs fed the streptomycin supplemented ration after attaining 100 lb of weight became dermatitic and lost weight constantly after 150 lb. Pigs continued on the unsupplemented corn-cottonseed meal ration from 100-200 lb gained 1.58 lb daily. The addition of penicillin to this ration resulted in an average daily gain of 1.78 lb.

Bacterial counts in the feces were significantly increased by the addition of penicillin but not by the addition of streptomycin or a combination of the two antibiotics. No significant correlation existed between total bacterial count and growth rate.

356. Luckey, T. D., 1956, Mode of action of antibiotics—evidence from germ-free birds. Pp. 135-145 in Proc. 1st International Conference on Use of Antibiotics in Agric. Published by Nat. Acad. Sci. and Nat. Research Council. Washington, D. C.

The growth response of germfree chicks fed antibiotic is:

Antibiotic	mg/kg fed	Growth Index	
	high/low	High level	Low level
None		100(*)	100(*)
Streptomycin	500/70	97(4)	117(3)
Bacitracin	35	97(6)	—
Oxytetracycline HCl	50/25	92(12)	117(14)
Procaine penicillin	46/11	96(8)	103(4)
Chloramphenicol	23	101(4)	

The number of germfree chicks used as controls for each experiment was equivalent to that used for each drug.

Positive growth response was also seen in experiments with germfree turkey poults.

357. Lanson, R. K. and Smyth, J. R., 1955, Pellets vs. mash plus pellets vs. mash for broiler feeding. *Poultry Sci.* 34, 234-235.

A 13% increase in the growth rate of pheasants to 4 weeks of age resulted from the addition of 10 ppm procaine penicillin to a mixed animal vegetable protein ration. Feed efficiency of the antibiotic fed group was somewhat improved. A number of birds developed a type of leg weakness, but in a number of cases, recovery occurred spontaneously. Incidence of this abnormality was less in the antibiotic fed group.

358. Ferrando, R., 1956, Précis d'alimentation du poulet. Du possin à la poule ponduese (Chicken nutrition. From the chick to the laying hen). Vigot Frères, Paris.

The author repeatedly noticed that antibiotics added to a ration containing a level of about 20% proteins produced in certain animals, after five to six weeks, deformities of the joints, legs and wings, but without affecting growth. These effects disappeared as soon as the protein level was reduced by two to three points.

359. Reynold, W. M., Warden, W. K. and Luther, H. G., 1953, Antibiotics, vitamin K and blood-clotting time in poultry. *Antibiotics Ann.* **1953-54**. Proc. Symposium on Antibiotics, Washington, D. C., pp. 380-385.

The results indicate that the levels of antibiotics used had no unfavorable effect on blood-clotting time compared to the control groups regardless of the level of vitamin K in the ration. Although no severe hemorrhaging was encountered in the controls, a vitamin K deficiency was apparent as demonstrated by prolonged blood-clotting times. Normal blood-clotting time resulted when 2.0% alfalfa or 0.18 mg of menadione was added to the ration.

In another trial, breeding hens fed for nine months on an all-mash breeder diet containing 50 to 1,000 gm of oxytetracycline or 500 gm diamine penicillin showed normal blood-clotting time.

360. O'Dell, B. L., Regan, W. O. and Hogan, A. G., 1957, Chlortetracycline in the nutrition of guinea pigs. *Proc. Soc. Exptl. Biol. and Med.* **96**, 553-555

Chlortetracycline HCl at a level of 25 mg/kg was beneficial in the breeding colony. It reduced number of abortion, adult mortality and cervical lymphadenitis. No effect on growth at levels of 25-200 mg/kg.

361. Snell, J. F., Thanassi, F. Z. and Sypowicz, D. A., 1958, Studies in Metabolic Spectra. I. Mode of action of tetracycline antibiotic. *Antib. and Chemo.* **8**, 57-75.

Promising methods for study of mode of action are being developed.

362. Pasteur, L., 1885. Observations relative à la note de M. Duclaux. *C. R. Acad. Sci.* **100**, 68.

363. Nencki, M., 1886, Bemerkungen zu Cives Bemerkung Pasteurs. *Schmiedeberts Arch. f. expt. Pathol. Pharmacol.* **20**, 385-388.

364. Metchnikoff, E., 1901, Sur la flore du corps humain. *Manchester Library and Philosophical Soc.* **45**, (5) 1-38.

365. Osborn, T. B. and Mendel, L. B., 1911, *Carnegie Inst. Bull.* **156**, parts 1 and 2. Washington, D. C.

Feces from normal rats prevented or corrected weight loss in deficient rats.

366. Gordon, W. S. and Taylor, J. H., 1954, The growth-promoting effect of antibiotics and their possible modes of action. *Proc. Royal Soc. Med.* **47**, 744-747.

Penicillin is absent in the cecum or large intestine even when fed at higher-dose levels, whereas aureomycin is detectable throughout the digestive tract. This fact suggests that the effect on the intestinal flora is confined to the organisms in the upper part of the digestive tract. Thus, the theory of an antimicrobial action of the antibiotics fed for growth promotion seems now less acceptable than that of control of sub-clinical disease.

367. Freerksen, E., 1956, Fundamentals of mode of action of antibiotics in animals. First International Conference on Antibiotics in Agriculture.

National Academy of Sciences and National Research Council 1956, Washington, pp. 91-105.

368. Eyssen, H., DeSomer, P. and Con Dijck, P., 1957, Further studies on antibiotic toxicity in guinea pigs. *Antibiot. Chemo.* **7**, 55-64.

Most antibiotics are toxic to guinea pigs. They cause a destruction of the normal intestinal flora which seems to result in deficiency of an essential growth factor and anabolic failure.

369. Phillips, B. P., Wolfe, P. A., Rees, C. W., Gordon, H. A., Wright, W. H. and Reyniers, J. A., 1955, Studies on the amoeba-bacteria relationship in amebiasis. Comparative results of the intracecal inoculation of germ-free, nomocontaminated and conventional guinea pigs with *Endamoeba histolytica*. *Am. J. Tropical Med. and Hyg.* **4**, 675-692.

370. Romoser, G. L., Shorb, M. S. and Combs, G. F., 1953, Effect of orally administered penicillin resistant microorganisms on growth of chicks. *Proc. Soc. Exper. Biol. and Med.* **83**, 17-21.

Pure cultures of *E. coli* and *A. aerogenes* were grown, lyophilized and fed to chicks as dietary supplements both in the presence and in the absence of procaine penicillin G. Little or no chick growth response was obtained when either of these organisms were added to the ration in the absence of the antibiotic. Greater gains were obtained when 10 ppm procaine penicillin G were fed. When viable cultures of *A. aerogenes* and *E. coli* were fed in combination with penicillin, growth was further increased significantly. The effectiveness of the antibiotic in promoting chick growth was increased 64 to 80% when these organisms were added to the feed. The results obtained illustrate the influence of bacterial environment on the antibiotic growth effect and in nutritional studies.

371. Anderson, G. W., Slinger, S. J. and Pepper, W. F., 1953, Bacterial cultures in the nutrition of poultry. *J. Nutrition* **50**, 35-46.

Supplementing a diet for chicks with certain microorganisms isolated from ceca of chicks fed penicillin resulted in improved weight.

372. Anderson, G. W., Slinger, S. J., Pepper, W. F. and Hauser, M. M., 1953, Bacterial cultures in the nutrition of poultry. II. Effect of dietary coliform cultures on the growth and cecal flora of poults. *J. Nutrition* **50**, 47-57.

Broad breasted Bronze poults were fed a practical diet supplemented with certain coliform cultures isolated from the cecal contents of chicks receiving penicillin. Feeding a mixed coliform culture of the viable cells from this culture resulted in improved weight of the poults when the diet contained penicillin. The weight increases were not significant at the 5% point. Neither the killed organisms nor the filtrate from this culture influenced poult weight. The results suggest that there was interaction between the viable mixed coliform organisms and penicillin.

373. Romoser, G. L., Shorb, M. S. and Combs, G. F., 1952, *Poultry Sci.* **21**, 932.

374. Anderson, G. W., Slinger, S. J., and Pepper, W. F., 1950, Bacterial cultures in the nutrition of poultry. I. Effect of dietary bacterial cultures on the growth and cecal flora of chicks. *J. Nutr.* **50**, 35.

375. Williams, W. L. and Taylor, R. R., Stokstad, E. L. R. and Jukes, T. H., 1951, Mechanism of the growth-promoting effect of aureomycin in chicks. *Fed. Proc.* **10**, 270.

Antitoxins of a variety of clostridia were without effect on the growth of control or aureomycin fed chicks.

376. Lindgren, N. O., 1954, Studies on the growth promoting action of antibiotics in poultry nutrition, 3. *Nord. Vet. Med.* **6**, 701-706. (*Nutrition Abstr. and Rev.* **25**, 1448, Jan. 1955.)

A second group received the same diet with a dietary supplement of *Bacterium coli* as previously used (Proc. 15th Internat. Vet. Congr. 1953. 913) and finished with a mean weight of 228 gm. Further groups that received, respectively, 5 ppm procaine penicillin, penicillin and *Bact. coli*, and 5 ppm neomycin and *Bact. coli*, weighed 246, 255 and 254 gm. The failure to obtain a growth stimulus with a dietary supplement of *Bact. coli* alone is in disagreement with the author's earlier findings.

Serial samples of droppings and of crop contents were taken from the birds in the different groups, and serial dilution, plating and counting methods were used to measure their content of coliform organisms. The day-to-day variations were extremely high and the counts bore no obvious relation to the diets.

377. Cook, F. D., Jowsey, J. R., Blakely, R. M. and MacGregor, H. I., 1955, The effect of massive doses of *Escherichia coli* in the feed of turkey poults, on growth and intestinal microflora. *Poultry Sci.* **34**, 1188.

In the present experiment massive doses of a pure culture of *E. coli* were rubbed into the feed daily.

The cultures either alive or autoclaved had no effect on growth to four weeks in the presence of procaine penicillin in the diet. In the absence of procaine penicillin only live cultures were fed. These had no effect on growth.

378. Kratzer, F. H., Grau, C. R., Starr, M. P. and Reynolds, D. M., 1951, Growth-promoting activities of antibiotics and yeast cultures for chicks and turkey poults. *Fed. Proc.* **10**, 386.

Streptomycin and other antibiotics increased the growth rate of poults. Certain strains of yeast also gave increased growth rate when fed at 1% level. Droppings fed to young chicks gave a growth depression which was overcome by aureomycin, autoclaved droppings produced none.

379. Smyser, C. F., Cleverdon, R. C., Kulp, W. L. and Materson, L. D., 1952, Effect of dietary antibiotics on number of *Clostridium perfringens* in feces of chickens. *Antibiotics and Chemother.* **2**, 363.

Cl. welchii increased in number when penicillin was fed to chicks.

380. Williams, W. L., Taylor, R. R., Stokstad, E. L. R. and Jukes, T. H., 1951, *Fed. Proc.* **10**, 270.

Mechanism of the growth promoting effect of aureomycin in chicks.

Fed both the culture and toxins from *Cl. welchii* to chicks and failed to produce growth depression.

381. Williams, W. L. and Taylor, R. R., Stokstad, E. L. R. and Jukes, T. H., 1951, Mechanism of the growth-promoting effect of aureomycin in chicks. *Fed. Proc.* **10**, 270.

Antitoxins of a variety of clostridia were without effect on the growth on control of aureomycin fed chicks.

382. Elam, J. F., Jacobs, R. L., Fowler, J. and Couch, J. R., 1954, Effect of dietary clostridia upon growth-promoting responses of penicillin. *Proc. Soc. Exptl. Biol. New York*, **85**, 645.

383. Lev, M., Briggs, C. A. E. and Coates, M. E., 1957, The gut flora of the chick. 3. Differences in cecal flora between "infected," uninfected and penicillin fed chicks. *J. Animal Sci.* **16**, 364-372.

Chicks reared in clean quarters did not have *Cl. welchii* in their intestinal tract. This organism was eliminated in the gut of chicks in dirty quarters by feeding penicillin.

384. Elam, J. F., Jacobs, R. L., Fowler, J. and Couch, J. R., 1954, Effect of dietary clostridia upon growth-promoting responses of penicillin. *Proc. Soc. Exptl. Biol. and Med.* **85**, 645-648.

The feeding of penicillin stimulated growth and decreased the clostridia count per gram of feces in birds maintained in old quarters. Penicillin failed to stimulate growth or decrease the clostridia count in birds maintained in clean quarters where the clostridia population was low. The feeding of fecal clostridia decreased the growth rate of birds reared in clean quarters. This decrease was overcome by addition of penicillin to the diet. The feeding of fecal clostridia to birds reared in old quarters where the clostridia population was high, failed to depress growth. Penicillin produced a growth stimulation only in cases where there was a decrease in the clostridia count per gram of feces. This indicated that one explanation for antibiotics stimulating growth is through an action on the anaerobic (clostridia) microflora.

385. Quinn, L. Y., 1955, Effect of antibiotic feeding on the intestinal microflora. Congr. Intern. Biochim. Resumes communs., 3 Congr., Brussels, 117-118 (Proc. 3rd Intern. Cong. Biochem., Academic Press, N. Y., 1955, 452-455).

Dietary chlortetracycline increased the *Aspergillus flavus-oryzae* content of feces. Culture of *Aspergillus flavus-oryzae* as well as concentrates of its growth factor stimulated the growth of young swine and chicks to much the same extent as feeding with the drug.

386. Anderson, G. W. and Slinger, S. J., 1957, Growth-promoting feed supplements. U. S. Patent No. 2,809,112, Oct. 8. (*Chem. Abstr.* **52**, 1508 1958.)

A growth-promoting supplement for poultry feed consists of the following: a dry, solid carrier such as soybean meal, corn meal, or other ground cereal, fuller's earth, oyster shells, etc., an antibiotic (penicillin or procaine penicillin); and a culture of a viable growth-promoting microorganism (*Escherichia coli*, a typical *E. coli*, or their mixture). Diets containing only penicillin addition to the basal diets give weights significantly better than the basal diet. The living cells alone cause a significant growth depression in the males, but in the presence of penicillin result in growth in males superior to that obtained with penicillin alone. This suggests interaction between the living cells and the antibiotic. No interaction is apparent between the dead cells and the antibiotic.

387. François, A. C., 1956, First International Conference on Antibiotics in Agriculture. National Academy of Science and the National Research Council, Washington, 1956, pp. 84-86.

Discussion of work from his laboratory.

Low levels of aureomycin failed to give any growth response when fed to chicks in a new laboratory. When 100 ppm were fed, the chicks grew at a faster rate than did control chicks.

388. François, C. and Michel, M., 1955, Action de la penicillin et de l'aureomycine sur les propriétés desaminantes de la flore intestinale du porc (Effect of penicillin and aureomycin on the deaminating properties of the intestinal flora of the pig). *C. R. Acad. Sci.* **240**, 124-126. (*Nutrition Abstr. and Rev.* **25**, 3996, July 1955.)

A sample of flora obtained by fractional centrifuging of pig intestinal contents was cultured *in vitro*. Liberation of ammonia was inhibited by addition of aureomycin or penicillin. A culture obtained from pigs fed on these antibiotics inhibited deamination in glutamic and aspartic and lysine and aspartic acid, arginine, citrulline, ornithine, histidine, β -alanine. The addition of aureomycin to a culture obtained from control pigs also inhibited deamination of these acids (with DL-alanine substituted for β -alanine), but penicillin was less active and did not inhibit deamination of aspartic acid, ornithine and DL-alanine.

389. Michel, M. and François, A. C., 1955, Relation entre l'influence des antibiotiques sur la croissance du porc et l'inhibition des desaminases de la flore intestinale (Relationship between the influence of antibiotics on growth of pigs and inhibition of deaminases of the intestinal flora). *Compt. rend. Acad. Sci.* **240**, 808-810.

It seems that (to these authors at least) the antibiotic effect is partly due to the prevention of a state of toxicosis in the young animal caused by the liberation of ammonia by the bacterial deaminases of the intestine. This hypothesis is confirmed *in vitro* by the inhibition of arginine and citrullin deaminase by the intestinal flora under the influence of antibiotics: terramycin, aureomycin, penicillin, streptomycin, bacitracin and chloramphenicol.

390. Febrier, R., François, A., Michel, M., Pero, R. and Salmon-Legagneur, E., 1955, (Antibiotics and growth.) *C. R. Acad. Agric. Fr.* **41**, 698-708. (Abstr. *J. Sci. Food and Agric.* **7**, 11-18, 11-19, July 1956).

The antibiotics, while having no effect on the utilization coefficient of food by the pig, inhibit deamination by intestinal flora, as shown by a direct relation between growth increase indices and deaminating effect for each antibiotic. CuSO_4 and 3-nitro-4-phenylarsonic acid have a similar effect on both deamination and growth. A mixture of aureomycin and chloramphenicol stimulates the growth and reduces the mortality of suckling pigs. Antibiotics, notably aureomycin, inhibit choline degradation by the bacterial flora of the digestive tract of the pig. Even if fed at double the normal rate the storage of antibiotics in pig tissues is feeble or nil.

391. Dintzis, R. Z. and Hastings, A. B., 1953, The effect of antibiotics on urea *Proc. Nat. Acad. Sci.* **39**, 571-578.

392. Smith, H. W. and Crabb, W. E., 1957, The effect of the continuous administration of diets containing low levels of tetracycline on the incidence of drug-resistant *Bacterium coli* in the feces of pigs and chickens; the sensitivity of the *Bact. coli* to other chemotherapeutic agents. *Vet. Rec.* **69**, 24-30.

A very much higher proportion of tetracycline-resistant *Bact. coli* were found in the feces of pigs and chickens which had been fed diets containing low levels of tetracyclines (4 to 30 gm/ton feed for 5 to 36 months) than were found in the feces of pigs and chickens kept on farms where these agents had never been fed.

Examination of fecal samples from pigs before and after commencing tetracycline feeding demonstrated the change that occurred from a predominantly sensitive to a predominantly resistant *Bact. coli* fecal flora. A high proportion of tetracycline-resistant *Bact. coli* were found in the feces of piglets whose mothers had been fed tetracyclines.

393. Coates, M. E., Dickinson, C. D., Harrison, G. F., Kon, S. K., Porter, J. W. G., Cummings, S. H. and Cuthbertson, W. F. J., 1952, A mode of action in chick nutrition. *J. Sci. Food and Agric.* 3, 43-48.

Chicks from the same batch in two other laboratories, where birds had not been kept before, grew equally well on the ration with and without penicillin, and growth was the same as that on the penicillin supplemented diet in the usual chick laboratory. The growth depression in the chicks maintained at the old laboratory site in the absence of dietary penicillin could not be attributed to differences in management or to recognizable disease. The authors suggest that the growth depression is due to an "infection" hitherto undescribed and shown to be transmissible and counteracted by penicillin.

394. Porter, J. W. G., 1957, Antibiotics and animal growth. *Symposia Soc. Exptl. Biol.* XI. The biological action of growth substances. Academic Press, Inc., New York, pp. 255-263.

A review of the role of the physical environment in the action of antibiotics upon chicks. Reasons for the systematic action seeming to be improbable are presented.

395. Coates, M. E. and Porter, J. W. G., The mode of action of antibiotics in chick nutrition. III. The nature of the "infection" counteracted by penicillin. *J. Sci. Food and Agric.* 6, 422-425.

Isolation units were designed to study the growth-despressing "infection" in chicks which is counteracted by penicillin. Preliminary results indicated the "infection" could be transmitted by feeding gut contents from "infected" chicks to newly-hatched chicks. Gut contents from freshly killed birds were added to the feed at the rate of 25 gm per cage of 5 birds.

The effect of penicillin on chicks raised in isolation boxes were given gut contents from "infected" and "uninfected" birds was investigated. Average weights at 12 days of age in the various treatment lots were as follows:

Treatment	Ave. wt. *(gm)
None	108
Penicillin	116
"Uninfected" gut contents	106
"Uninfected" gut contents + penicillin	104
"Infected" gut contents	85
"Infected" gut contents + penicillin	102

396. Whitehair, C. K. and Thompson, C. M., 1956, Observations on raising "disease-free" swine. *J. Am. Vet. M. A.* 128, 94-98.

Baby pigs collected by cesarotomy and fed a basal diet were given 10 mg of Aureomycin per pound of feed as a supplement. By the end of the 28 day experiment period, it was seen that the antibiotic supplement did

not improve growth performance or feed efficiency. It is believed that the growth-promoting effect of antibiotics for young pigs results, at least in part, from the control of infections or their secondary complication.

397. Hill, E. G. and Larson, N. L., 1955, Effect of chlortetracycline supplementation on growth and feed utilization of the unsuckled baby pigs obtained by hysterectomy. *J. Animal Sci.* **14**, 1116-1121.

Pigs delivered by hysterectomy into clean isolated units and fed pasteurized milk and egg yolk grew at faster rate when chlortetracycline was added to the diet.

398. Landagora, F. T., Rusoff, L. L. and Harris, B., Jr., 1957, Effect of aureomycin on young dairy calves raised in a new environment. *J. Dairy Sci.* **40**, 50-55.

The effect of oral fed (50 mg daily in milk and 0.5% Aurolac 2A in feed) and intramuscular-injected (400 gm daily) aureomycin on young calves raised in a new uncontaminated and in an old environment was investigated. The data shows that regardless of what type of environment calves are raised in, aureomycin administration statistically ($P < 0.01$) increased body weight gains and feed utilization efficiency at 12 weeks of age. An earlier stimulation in growth was observed in calves raised in a new barn than in those raised in an old barn. The growth response from antibiotics administration was detected earlier in the oral-fed groups than in the injected-treated animals. In the new barn, the growth-promoting effect of aureomycin administration was detected as early as two weeks, whereas in the old barn the effect was not observed for four weeks. This finding indicates that the cleanliness and sanitary conditions of the environment favor a greater and more effective growth-stimulation response of aureomycin feeding.

399. Briggs, G. M., 1950, Antibiotics in poultry ration. *Feedstuffs* **22**, 32-36.

The term "promotant" is suggested and defined as any substance, or agent, which promotes a desirable effect, such as faster growth or better feed efficiency or improved reproduction, by its action on the intestinal flora when added to the diet of non-ruminating animal. It may be a drug, a vitamin (when the action is indirect in this manner), or any other type of compound. It includes, among others, the antibiotics, the phenylarsonic acid derivation and several of the sulfa drugs.

400. Johnson, W. P. and Algeo, J. and Kleck, J., 1957, The effect of chlortetracycline supplementation on the incidence of foot rot and feed lot performance in cattle. *Vet. Med.* **52**, 375-378.

Chlortetracycline significantly reduced the necessity for treatment of disease from all causes—240 control animals required treatment as compared with 36 in the supplemented groups. In an outbreak of foot rot, 172 control animals were affected while only two of the supplemented animals showed symptoms. In all diseases other than foot rot, 68 control animals required treatment and only 34 chlortetracycline-supplemented animals required treatment.

- 400a. Brown, L. R., Johnson, R. H., Jacobson, N. L. and Homeyer, P. G., 1958, Effect of administration of oils and penicillin on incidence and severity of bloat and certain other responses in cattle. *J. Animal Sci.* **17**, 374-385.

Procaine penicillin (75 mg/day) reduced bloat in steers fed alfalfa

silage when first fed but later no difference was seen between these and control fed no penicillin.

- 400b. Huey, C. R. and Edwards, P. R., 1958, Resistance of *Salmonella typhimurium* to tetracyclines. *Proc. Soc. Exptl. Biol. and Med.* **97**, 550-551.

No cultures taken prior to 1948 were resistant while 9% of recent cultures from fecal and 5% of recent cultures from man were resistant.

401. Bartley, E. E., Fountaine, F. C., Atheson, F. W. and Fryer, H. C., 1953. *J. Dairy Sci.* **37**, 259.

Antibiotics in dairy cattle nutrition. III. Effects of different levels of aureomycin intake upon the growth and well being of dairy calves and the association of differences with changes in environment.

402. Korpp, B. N., 1957, The effect of penicillin on survival of mice receiving lethal doses of trypan blue. *Antib. and Chemo.* **7**, 135-139.

Penicillin prolonged survival time of mice given lethal doses of trypan blue.

403. Luther, H. G. and Brown, J. H., 1951, Antibiotics in ration of hogs—effect of withdrawal and long term feeding, levels and comparison of antibiotics including anti-fungal type. *J. Animal Sci.* **10**, 1055.

In these tests terramycin was included in the gestation, lactation and creep rations at a level of 8 grams per ton. The results show that under the controlled dry lot feeding conditions the growth stimulation from terramycin supplementation was essentially unchanged, giving 20 to 45% increase in growth at eight weeks, 8 to 15% at market weight. Rimocidin, a new antibiotic effective against yeast and mold, but ineffective against bacteria, gave a 51% increase in rate of growth at eight weeks, an observation which complicated some prevailing theories on mode of action in promoting increased rate of growth.

404. Dick, E. C. and Johanson, K. R., 1957, Effects of oral and parenteral administration of degraded and active antibiotics to rats fed a vitamin B₁₂ deficient ration. II. Indirect evidence for an extra-intestinal cause of growth stimulation by antibiotics. *Antib. and Chemo.* **7**, 349-358.

Little correlation was noted between the intestinal concentration of antibiotics and growth enhancement. Parenteral administration was as effective as oral. The insoluble antibiotic, carbomycin B, gave growth stimulation with no change in intestinal microorganisms when given peritoneally but had no growth effect given orally.

405. Landagora, F. T., Rusoff, L. L. and Harris, B., Jr., 1957, The effect of chlortetracycline on carcass yields including physical and chemical composition of dairy calves. *J. An. Sci.* **16**, 654-661.

Injected chlortetracycline (400 mg I. M. per wk.) increased the growth rate and food efficiency in dairy calves as did those fed 50-75 mg daily. Heavier carcass weights and dressing percentages were noted. Increased bone and muscle weights indicated general growth stimulation.

406. Bruggemann, J., 1956, First International Conference on Antibiotics in Agriculture. National Academy of Sciences and National Research Council, Washington, pp. 149-152.

Parenteral administration of antibiotics to rats produced a growth stimulation.

407. Elam, J. R., Jacobs, R. L., Tidwell, W. L., Gee, L. L. and Couch, J. R., 1953, Possible mechanism involved in the growth-promoting responses obtained from antibiotics. *J. Nutrition* **49**, 307-317.

The addition of penicillin to the basal diet (orally or parenterally) produced an increase in growth. Inactivated penicillin (parenterally) produced a similar increase, but failed to do so when given orally. Aureomycin, bacitracin and combinations of penicillin and aureomycin or penicillin and bacitracin also increased growth.

Administration of penicillin (orally or parenterally) inactivated penicillin (parenterally), and combinations of penicillin and aureomycin, and bacitracin and penicillin produced a very significant decrease in the total number of fecal clostridia. The decrease in clostridia produced by feeding inactivated penicillin was also significant, but was of a lesser magnitude than that which occurred through the injection of inactivated penicillin.

The growth obtained in the second experiment was not equal to that obtained in the first trial.

408. Elam, J. F., Gee, L. L. and Couch, J. R., 1951, Function and metabolic significance of penicillin and bacitracin in the chick. *Proc. Soc. Exptl. Biol. and Med.* **78**, 832-836.

Parenteral administration of antibiotics or autoclaved penicillin solution increased the rate of growth with little effect on the fecal aerobic microflora.

409. Fell, R. V. and Stephenson, E. L., 1953, The effect of penicillin and penicillamine on chick growth when injected and when fed orally. *Poultry Sci.* **32**, 1092.-1093.

The results were as follows: In the first trial, penicillin injected at a level of 0.05 mg per day and penicillamine at the 0.1 mg per day level resulted in growth responses which were significant ($P = 0.05$). The injection of 0.05 mg of penicillamine per day resulted in a growth response which approached significance. In a second trial, penicillamine injected at both the 0.05 mg and 0.1 mg levels resulted in growth responses which were highly significant ($P = 0.01$). In this trial, the injection of penicillin did not result in a significant growth response, but the oral feeding of penicillamine resulted in a response which approached significance. In both trials, the most consistent growth response was obtained from penicillamine injected at a level of 0.1 mg per day.

410. Williams, W. L., Esposito, R. G. and Stokstad, E. L. R., 1953, Comparison of chick and microbiological methods for assay of penicillin in feeds. *Fed. Proc.* **12**, 290-291.

411. Taylor, J. H. and Gordon, W. S., 1955, Growth promoting activity for pigs of inactivated penicillin. *Nature* **176**, 312-313.

Treatment	Growth rate lb/day	Feed conversion ratio
None	1.14	3.81
Procaine penicillin	1.25	3.56
Penicillin inactivated by heat	1.20	3.72
Penicillin inactivated by penicillinase	1.20	3.76
Penicillin inactivated by salts of heavy metals	1.22	3.61

In both experiments active penicillin produced a significant mean increase in growth rate of 14.3% and inactive material produced a significant mean increase in growth rate of 9.4%. The inactive material thus produced 66% of the growth response achieved with active penicillin. The difference between the effect of the active and inactive material was not significant.

412. Frost, D. V. and Spruth, H. C., 1956, Arsenicals in feeds. In Symposium on Medicated Feeds, by H. Welch. Medical Encyclopedia, Inc., New York, pp. 136-149.

Arsenicals appear to parallel all of the actions of antibiotics in animal growth, egg production, food efficiency and nutritive requirement performance except they are not bacteriostatic at low levels.

413. Berry, M. E. and Schuck, C., 1954, The effect of aureomycin on growth and protein utilization in the rat. *J. Nutrition* **54**, 271-284.

Nitrogen metabolism studies indicated that aureomycin improved the apparent digestibility of the cotton-seed and soybean-meal proteins, but impaired utilization following absorption. This effect was evident to such a degree in rats receiving cotton-seed meal diets that nitrogen retention expressed both in terms of per cent of ingested nitrogen and absorbed nitrogen was actually depressed in antibiotic-supplemented animals.

414. Bird, H. R., Groschke, A. C. and Rubin, M., 1948, Effect of arsonic acid derivatives in stimulating growth of chicks fed certain diets. *Federation Proc.* **7**, 283.

Diets high in soybean oil meal were improved by the addition of 0.005% 3-nitro-4-hydroxyphenyl arsonic acid. Higher and lower levels were less favorable. Phenylarsonic acid, p-hydroxyphenyl arsonic acid and m-nitro phenylarsonic acid were also active.

- 414a. Warden, W. K. and Schaible, P. J., 1958, Effect of gibberellic acid in broiler-starter rations. *Poultry Sci.* **37**, 490-491.

Lot	Gm/ton	Wgt (40 birds)
1	0	457
2	0.2	465
3	2	464
4	20	486

415. Ely, C. M., 1951, Chick-growth stimulation produced by surfactants. *Science* **114**, 523-524.

Lauryl ethylene oxide condensate consistently gave increased growth rate in chicks fed a practical diet. Increased feed efficiency was noted in one group.

416. March, B. E., Burdett, M. and Biely, J., 1954, Antibiotics and surface active agents in chick nutrition. *Poultry Sci.* **33**, 300-304.

Tween-80 (polyoxyethylene sorbitan mono-oleate) at levels of 1.0 and 2.0% in a mixed animal-vegetable protein starting diet for chicks for eight weeks gave a stimulus to growth at 8 weeks similar to that obtained with 0.15 gm procaine penicillin G per 100 lb of ration.

417. McDonald, M. W., 1956, Effects of detergents on growth of chickens. *Agric. Gaz. N. S. W.* **67**, 39-41. (*J. Sci. Food and Agric.* **8**, 1-189, May 1957.)

Addition of 0.5% detergent (60% Na tetrapropylene-benzenesulfonate) to the diet of chicks stimulated growth to 12 weeks of age. The treatment was more effective when 6.6 ppm of penicillin was also added. The detergent was not as effective as was penicillin in controlling diseases. In another test the detergent produced no growth response under conditions in which penicillin failed to produce a response.

- 417a. Balloun, S. L., 1955, The effect of quaternary ammonium derivatives in chick diets. *Poultry Sci.* **34**, 191-196.

Quaternary ammonium salts consistently improved feed efficiency and growth in old environments.

- 417b. Ney, L. F. and Newell, G. W., 1954, The effects of a sodium alkyl axyl sulfonate detergent on the growth of chicks. *Poultry Sci.* **33**, 297-299.

Growth was increased 6-11% in a 12 week experiment with chicks fed surfactant.

- 417c. Almquist, H. J. and Merritt, J. B., 1955, The effects of a detergent in the diet of range turkeys. *Poultry Sci.* **34**, 740-741.

Surfactant increased the feed efficiency in turkey poults. A very slight improvement in growth was also noted when the poults ate grain.

- 417d. Lillie, R. J., Sizemore, J. R. and Denton, C. A., 1958, A study of surface active agents in broiler diets. *Poultry Sci.* **37**, 288-292.

A combination of surfactants gave consistent slight improvements in the growth of chicks. Food efficiency was also improved. Antibiotic and surfactant effects were not additive.

418. Anderson, G. W., Cunningham, J. D. and Slinger, S. J., 1952, Effects of terramycin and certain phenylarsonic acid derivatives on the growth and intestinal flora of turkey poults. *J. Nutrition* **48**, 539-552.

Supplementation with terramycin alone or in combination with any of the phenylarsonic acid derivatives resulted in weight increases which were highly significant at 30 days of age. In the absence of terramycin, highly significant weight increases resulted from the inclusion of magnesium 4-hydroxyphenylarsonate and 3-nitro-4-hydroxyphenylarsonic acid. In general, significant increases in weight were accompanied by slight improvement in feed efficiency.

419. Schendel, H. E. and Johnson, B. C., 1952, Effect of antibiotics, arsanilic acid, surfactants, and sulfas on the growth of baby pigs fed synthetic diets. *J. Animal Sci.* **11**, 775.

Aureomycin and terramycin exerted the greatest effect on growth. Penicillin, streptomycin or chloromycetin also stimulated growth while rimocidin failed to produce a significant growth response. Under our conditions we have been unable to demonstrate a response of increased growth exerted by the surface activating agents, either alone or as a mixture. We have studied ethomeen C/15 alone and a mixture of ethofat C/15, ethomid C/15, arquad S, aerosol SE, aerosol OS, and ultra-wet K. Neither of the sulfa drugs used in this study, sulfathalidine and sulfisoxazole (ganstrisin), were shown to stimulate the growth of baby pigs. Arsanilic acid did increase the rate of growth significantly.

420. Luecke, R. W. and Hoefer, J. A., 1956, Antibiotics and sulfaquinoxaline fed from weaning to market. 1st Ann. Michigan Swine Day, Article 2, pp. 4.

Feeding the coccidiostat sulfaquinoxaline at 50 gm/ton resulted in a 10% increase in growth rate over the control lot. There is no evidence to indicate that coccidia are a problem in swine, but on the other hand there is no adequate explanation for the growth stimulation of sulfaquinoxaline.

The combination of sulfaquinoxaline and streptomycin at two levels proved to be slightly superior to the sulfaquinoxaline alone at the 50 gm level.

421. Luecke, R. W., Hofer, J. A. and Thorpe, F., Jr., 1952, The growth-promoting effect on pigs of a surface-active agent. *Quart. Bull. Mich. Agr. Expt. Sta.* **34**, 331-332.

Pigs fed aureomycin or ethomoid C/15 (a polyoxyethylene N substituted fatty acid amide) grew faster than control pigs. The combination gave somewhat less response than either singly.

422. Barber, R. S., Braude, R. and Michell, K. G., 1955, Antibiotic and copper supplements for fattening pigs. *British J. Nutrition* **9**, 378-381.

The high-copper mineral mixture and aureomycin, either alone or together, and the copper-sulphate supplements were equally effective in significantly increasing the rate of gain of the pigs. None of the treatments had any significant effect on the commercial bacon factory grading of the carcasses.

423. Barber, R. S., Braude, R., Mitchell, K. G. and Rook, J. A. F., 1956, Further studies on antibiotic and copper supplements for fattening pigs. *Proc. Nutrition Soc.* **15**, ix-x.

Supplements of either copper sulfate (0.1%), oxytetracycline (terramycin) (10 gm/ton) or aureomycin (20 gm/ton) were equally effective in significantly increasing growth rate. All the supplements improve the efficiency of food utilization but only with aureomycin did the effect, attain the 5% level of significance. The rate of food consumption (lb/day) was significantly increased by all three supplements.

424. Wallace, H. D., Ney, W. A. and Cunha, T. J., 1951, Various antibiotics and 3-nitro-4-hydroxyphenyl arsonic acid in corn-peanut meal rations for swine. *Proc. Soc. Exper. Biol. and Med.* **78**, 807-808.

Terramycin and the two levels of aureomycin resulted in an increase in gains over the control lot and all other treatments. Although the arsonic acid derivative did not increase the growth rate, feed efficiency was considerably better than for all other lots.

Both aureomycin and terramycin controlled an intermittent type of diarrhea observed on the basal rations. The other supplements were not effective in this respect.

425. Schendel, H. E., 1954, The mechanism of action of antibiotics in stimulating growth in animals on marginal or deficient diets. *Dissert. Abstr.* **14**, 1490. (Abstr. *J. Sci. Food and Agric.* **6**, 1-191).

Using baby pigs 48 to 96 hours old and fed a synthetic diet, the antibiotic Terramycin and No. 802 increased gains significantly. Arsanilic acid was observed to produce significant increase in gains when fed at a level of 90 mg/kg of dry matter of diet. Rimocidin, sulfisoxazole and nine surface-active agents failed to affect gains. Aureomycin also failed to stimulate the growth of a piglet fed a reconstituted skim milk diet. Data suggest that the effect of terramycin (found to increase

growth significantly) on the total number of microorganisms in all intestinal sections from the duodenum to the cecum is to increase their number about tenfold.

426. Wing, J. M., 1957, Effect of para amino salicylic acid and chlortetracycline alone and in combination on dairy calves. *J. Animal Sci.* **16**, 854-857.

Results indicate that the addition of chlortetracycline or PAS alone stimulated gains in young calves but a combination of the two drugs resulted in growth which was not significantly different from that of comparable controls.

- 426a. Russo, J. M., Hanson, L. E. and Jezeski, J., 1954, The effect of aureomycin and arsanilic acid on nitrogen balance in pigs. *J. Animal Sci.* **13**, 998.

Arsanilic acid or chlortetracycline reduces the nitrogen excretion in pigs.

427. Schole, J., 1953. The significance of biological oxidation-reduction systems. *Naturwiss* **40**, 555 (*C. A.* **48**, 8908).

Therapeutic doses of penicillin give an alarm reaction. As antibiotics inactivate SH systems, they also give hypertrophic changes in adrenal cortex.

428. Calesnick, B., Harris, W. D. and Jones, R. S., 1954, Antithyroid action of antibiotics. *Science* **119**, 128-129.

All the treated groups showed an increase in size of the thyroid and a marked depression of the uptake for L^{131} . The changes induced by the antibiotics were not as great as by the thiouracils. In one of the Aureomycin fed rats, evidence of hyperplasia was noted following histological examination; no changes were observed in the penicillin-fed group. Vascularity after iodothiouracil was far less than after propylthiouracil.

429. O'Dell, B., 1955, Personal communication, April 23.

430. Palafox, A. L. and Rosenberg, M. M., 1952, Differential growth response of male and female chicks to antibiotic supplementation. *Poultry Sci.* **31**, 1110-1111.

Male chicks maintained in battery brooders and fed from 2 to 42 days of age a mixed animal vegetable protein ration supplemented with aureomycin and B_{12} grew 10.3% faster than their controls. Those chicks receiving a terramycin supplement grew 4.0% faster than their respective controls. Male chicks gave a greater response than female birds.

431. Almquist, H. J. and Merritt, J. B., 1953, The value of antibiotic supplements for growth and feed conversion in diets for growing turkeys. *Poultry Sci.* **32**, 878-880.

The addition of 2 gm of diamine penicillin per ton of a commercial mash-grain ration proved consistently beneficial with respect to gains and feed efficiency to toms and from 1 to 110 days of age. The same was true for turkey hens from 1 to 80 days of age. However, in the hens, no accelerated growth rate was evident in the period from 80 to 110 days of age although feed efficiency continued to improve. Birds on mash from which the antibiotic supplement was withdrawn reverted to gains and feed conversions similar to those of the control groups.

432. Begin, J. J. MacLaury, D. W., Risner, R. and Insko, W. M., Jr., 1951, Breed, sex and age variation among chicks in response to antibiotic supplementation. *Univ. of Kentucky Bull.* 597, pp. 12.

When the 10 week weights of the New Hampshire males and females on the bacitracin ration are compared, a definite sex interaction is observed. The male growth was found to be highly significant as compared to the basal, while the females failed to respond significantly to bacitracin supplementation.

433. Slinger, S. J., Pepper, W. F. and Hill, D. C., 1952, Effect of penicillin on the tolerance of turkey to fat. *Arch. Biochem. and Biophysics* 37, 266-269.

Under the conditions of this experiment, the energy requirement of turkeys for maximum growth and feed efficiency was in excess of 82 cal/lb. The addition of 10% corn oil to the diet, raising the energy content to 908 cal/lb, caused growth retardation. Penicillin supplementation relieved considerably the growth-depressing effect of the high level of fat in female poults.

434. Iyenger, M. R. S. and Starkey, R. L., 1953, Synergism and antagonism of auxin by antibiotics. *Science* 118, 357-8.

Oxytetracycline, chloramphenicol, and streptomycin stimulated greater elongation of a section of *avena* coleoptiles in the presence of indoleacetic acid.

435. Goodman, R. N. and Hemphill, D. D., 1954, The effects of indole-3-acetic acid on the plant disease-inhibiting properties of antibiotics. *Science* 119, 347-348.

Indole-3-acetic acid increases the effectiveness of antibiotics (with penetrants) in control of *Erwinia amylovora*.

436. Pearl, R. and Parker, S., 1922, New experimental data on the influence of density of population upon duration of life in *Drosophila*. *Am. J. Hyg.* 3, 94. 122 or *Am. Naturalist* 38, 812, 1922.

437. Cohen, A. M. and Rachnilewitz, M., 1953, Effect of aureomycin in rats with chronic alloxan diabetes. *Proc. Soc. Exptl. Biol. and Med.* 83, 50.

Since patients with severe diabetes had shown improvement during antibiotic treatment, chlorotetracycline was fed to rats with alloxan diabetes. The antibiotic doubled the growth rate and increased the urinary sugar.

438. Baez, S., Srikantla, S. G., Forbes, I. and Shorr, E., 1955, Metabolic action of aureomycin on liver; inhibition of anaerobic release of ferritin (DVM *Fed. Proc.* 14, 6-7.

Greater survival of rats fed chlortetracycline after a rotating drum treatment suggested the liver of the rats should be examined for release of ferritin. The livers of rats fed the drug did not release ferritin in solution as did liver slices from untreated rats.

439. Fine, J., 1952, Effect of antibiotics on irreversibility to transfusion in hemorrhagic shock. *Harvard M. Alum. Bull.* 26, 105.

Penicillin and chlortetracycline were effective orally (but not parenterally) in giving a resistance to irreversible shock induced in dogs by hemorrhage.

440. Gustafsson, G. E. and Koletsky, S., 1951, Effect of oral terramycin prior to whole body x-radiation. *Proc. Soc. Exptl. Biol. and Med.* 78, 489-490.

Oxytetracycline protects against whole body x-radiation by reducing bacteremia.

- 440a. Zweifach, B. W., 1959, Hemorrhagic shock and germfree life. *Ann. N. Y. Acad. Sci.* In press.

441. Gyorgy, P., 1954, Antibiotics and liver injury. *Ann. N. Y. Acad. Sci.* **57**, 925-931.

The effect of antibiotics to increase the survival time of rats fed a diet low in proteins (9-10%), methionine and vitamin E was found to decrease with time (it gave 3 fold protection in 1949, and less than 2 fold in 1951). Despite the fact that the food intake was not increased, the animals grew much better when antibiotics were in the diet.

442. Luckey, T. D., Reyniers, J. A., Gyorgy, P. and Forbes, M., 1954. Germ-free animals and liver necrosis. *Ann. N. Y. Acad. Sci.* **57**, 932-935.

When germfree rats were fed restricted amounts of a diet low in protein, methionine and vitamin E, they developed hemorrhagic necrosis of the liver.

443. Baxter, J. H. and Campbell, H., 1952, Effect of aureomycin on renal lesions, liver lipid and tissue choline in choline deficiency. *Proc. Soc. Expt. Biol. and Med.* **80**, 415-19.

Chlortetracycline but not other antibiotics would prevent renal lesions in the rats fed a high fat, low choline diet.

444. Meites, J., 1952, Beneficial effects of vitamin B₁₂ and Aureomycin in rats given large doses of cortisone. *Proc. Soc. Exptl. Biol. and Med.*, **81**, 307-311.

Cortisone acetate alone induced complete inhibition of body growth, severe alopecia and marked atrophy of the thymus gland. Vitamin B₁₂ (200 mcg/kg diet) or aureomycin (0.005%) largely counteracted these inhibitory effects of cortisone acetate, and the combination of the two was more effective than either alone.

445. Carlson, C. W., Kohlmeyer, W., Hendrick, C. and Wilcox, R. A., 1956, Effects of energy and protein levels and antibiotics on growing turkeys. *South Dakota Agric. Exper. Sta. Techn. Bull.* **17**.

The addition of 2.4 mg of diamine penicillin or 5 mg of chlortetracycline to each pound of feed showed greater and more consistent advantages with low energy diets than in the presence of high energy levels. The value of antibiotics also was more pronounced under condition of environmental stress, such as cold weather. Chlortetracycline appeared to enhance carcass yield of hens with either low- or high-energy diets.

446. Scott, H. M., Goffi, E. A. and Glista, W. A., 1952, The protein requirement of the chick as influenced by aureomycin. *Poultry Sci.* **31**, 751-752.

Aureomycin did improve growth more at protein levels below the requirement than in the range of the requirement, but since the improvement in protein efficiency was no better than the improvement in overall feed efficiency, there is no evidence of a protein-sparing action of the antibiotic.

447. Yacowitz, H., 1953, Supplementation of corn-soybean oil meal rations with penicillin and various fats. *Poultry Sci.* **32**, 930.

The addition of 10 and 15% cottonseed oil retarded growth and caused a high incidence of feather picking; penicillin supplementation markedly reduced feather picking.

448. Hill, C. H., Keeling, A. D. and Kelly, J. W., 1957, Studies on the effect of antibiotics on the intestinal weights of chicks. *J. Nutr.* **62**, 255-267.

The small intestine decreased in weight before an increase was noted in body weight. Low levels of antibiotic affected intestinal length which had no effect on growth. High levels of penicillin counteracted the growth retarding effect of 10% raw soybean meal but not 30% raw soybean meal.

449. Jukes, T. H., 1956, Antibiotic in nutrition and stress. *Proc. Univ. Maryland 1956. Nutrition Conference for Feed Mfgs.*, pp. 32-33.

In certain instances it is possible to show that stresses of various types will lead to such effects as slow growth, evidence of diseases, lower egg production and high mortality and that these undesirable effects may be reduced by feeding a high level of a suitable antibiotic. An example of this is a condition that is produced in turkey poults by taking too long to get them from the hatchery to the brooder house. Another illustration is a drop in egg production that may be caused by unusually hot weather. A third example is the stress caused by chronic respiratory disease which appears to lower the resistance to intercurrent infection.

The effects of antibiotics on nutrition and stress indicate the importance of the bacterial environment as a factor in animal production even when recognizable diseases are not present.

450. Luckey, T. D., Gordon, H. A., Wagner, M. and Reyniers, J. A., 1956, Growth of germfree birds fed antibiotics. *Antibiotics and Chemother.* **2**, 36-40.

Growth data are reported for experiments in which antibiotics were fed to germfree chicks and turkey poults. Birds are fed a high vitamin semi-synthetic diet and the following relatively high levels of antibiotics: 23 mg/kg chloramphenicol, 35 mg/kg. bacitracin, 70 mg/kg ampicillin, 450 mg/kg streptomycin, 50 mg/kg oxytetracycline hydrochloride, and 46 mg/kg procaine penicillin. There was a slight consistent decrease in growth rate with these levels, indicating that lower levels of antibiotics should be fed. When the germfree chicks were fed 25 mg oxytetracycline/kg diet, they appeared to grow at a faster rate than did the control chicks fed no antibiotics. Germfree chicks fed 11 mg/kg procaine penicillin showed possible growth stimulation. Growth rate of germfree turkey poults was generally faster when they were fed procaine penicillin, 46 mg/kg diet.

451. Forbes, M. J., Parks, J. T. and Lev, M., 1959, Growth response and effect on intestinal flora in germfree and conventional animals fed antibiotics. *Ann. N. Y. Acad. Sci.* In press.

No growth response was obtained in germfree chicks and poults fed 25 ppm of procaine penicillin.

452. Richet, C., 1906-07, De l'action de doses Minuscules de Substance sur la Fermentation Lactique—troisième memoire—Periodes d'acceleration et de Ralentissement. *Archives Internationale de Physiologie* **4**, 18-50.

The curve of acceleration, retardation, and normal growth as shown by rate of fermentation of milk is shown. The hypothesis set forth is the formation of an active electric force.

With temperature and the other factors held constant, the author

- presented results on AgNO_3 , ThNO_3 , thorium Cl, PtCl , CoCl_2 , NMCl , LiCl_2 , BaCl_2 , VaCl_2 , and a mixture of Ag, Co, Li, Mn, Pt, and thorium.
53. Garrison, F. H., 1929, An introduction to the history of medicine. W. B. Saunders Company, pp. 437-438.
S. Hahnemann's first doctrine was a revival of the Paracelsian doctrine: *similia similibus curantur*; his second, that the dynamic effect of drugs is heightened by extreme dilution, and his third is that most chronic diseases are a manifestation of "Psora."
 54. Luckey, T. D., 1959, Modes of action of antibiotics in growth stimulation. *Proc. 7th Intern. Cong. Microbiol. Stockholm*, in press.
Any stimuli smaller than that which evokes no more than the minimum response will elicit a relatively greater response than it warrants. Thus imbalance in favor of the response is commonly engendered when minute stimuli are applied.
 55. Nageli, 1893, Ueber oligodynamische Erscheinungen in Lebenden Zellen. Neue Denkschrift, d. allgem. schweizer. Gesellsch., f.b. gesamte Naturwissenschaft, Bd. 33, I.
Very dilute solutions of metallic ions (Ag^+) were toxic to algae.
 56. Schulz, H., 1888, Ueber Hefegifte. *Arch. ges. Physiol.* **42**, 517-541.
Kleine Dosen reizen, grosse dozen lahmen—poisons are stimulants in small doses. Mercury, iodine, arsenic, chromium compounds gave growth stimulation in bacteria.
 57. Delepine, A. S. and Greenwood, A., 1914, *J. R. Sanit. Instit.* **35**, 317.
Cu, silver, Zn, Cd, Hg, ions had growth stimulation at low levels. (Topely and Wilson, p. 120, 3rd edition.)
 58. Winslow, C. E. A. and Hotchkiss, M., 1921, Studies on salt action. V. The influence of various salts upon bacterial growth. *Proc. Soc. Exper. Biol.* **19**, 314-315.
 PbCl_2 , TiCl_3 , SnCl_4 , NiCl_2 , CeCl_2 , HgCl_2 , CdCl_2 , NH_4Cl , SrCl_2 , NaCl and KCl were all stimulating at 1/10 to 1/5 of the concentration in which they were inhibitive. K and Na stimulate at 0.25 molar Ce or HcCl_2 was stimulating at 0.000005 molar. Pb was stimulating at 0.00005 molar.
 59. Hotchkiss, M., 1923, Studies on salt action. VI. The stimulating and effect of certain cations upon bacterial growth. *J. Bacteriol.* **8**, 141-162.
The majority of chlorides studied showed growth stimulation.
 60. Peterson, J. B., 1926, Mercurials: A proposed method of laboratory evaluation and classification. *J. Amer. Med. Assoc.* **87**, 223-225.
 CO_2 production in yeast was stimulated by mercury salts.
 61. Richet, C., 1905, De l'action de doses minuscules de substances sur la fermentation lactique. *Arch. Intern. de physiologie* **3**, 203-217.
Formaldehyde in minute quantities stimulated the fermentation.
 62. Feeney, R. E., Petersen, I. M. and Sahinkaya, H., 1957, "Liesegang-like" rings of growth and inhibition of bacteria in agar caused by metal ions and chelating agents. *Journ. of Bacteriology* **73**, 284-290.
Formation of multiple alternate rings of growth and inhibition of growth of microorganism on agar, caused by chelating agents and metal ions action compared with formation of Liesegang rings caused by interactions of salts in gelatin or agar.

463. Hume, S., 1909, Die begünstigende Reizwirkung Kleinsten Mengen Bacteriengiften auf die Bakterienvermehrung. *Zlb. Bakt. Abt. 1* 19, 135.

The following stimulated coli growth (parentheses indicate active concentration): Fluor (1-100,000), CuSO_4 (1-1,000,000), Thy (1-30,000), alcohol (1-1,000), ether (1-100), formaldehyde (1 million). Formaldehyde was also active for cholera, typhus and dysentery.

465. Fred, E. B., 1911, *Zentralb. f. Bakt. (Abt. 2)* 31, 185-245. Über die Beschleunigung der Lebenstätigkeit höherer und niederer Pflanzengruppen durch kleine Giftmengen.

466. Hofmann, P., 1922, Über die Gültigkeit des arndt-Schulzacher biologischen Grundgesetzes bei der Wirkung von Bacteriengiften Thesio Munich.

Disinfectants given in small amounts accelerate bacterial growth.

467. Branham, S. E., 1929, Effects of certain chemical compounds upon the course of gas production by bakers yeast. *J. Bact.* 18, 247-264.

The effect of individual chemicals is characteristic and commonly observed. Sees stimulation of fermentation by small amounts of germicides.

468. Lamanna, C., 1942, Growth stimulation by sulfanilamide in low concentration. *Science* 95, 304-305.

Zone of heavy growth between that of inhibition and that of normal growth seen in bacteria and yeast.

469. Johnson, F. H., 1943, Mechanism of p-aminobenzoic acid action and the parallel effects of ethyl carbamate (urethane). *Science* 95, 104-105.

PAB-sulfa ratio is 1/23,000; too great for acidic metabolite action so must be a narcotic action. Urethane also counteracts sulfa drug. Sulfa drug, PAB or urethane all stimulate growth and luminescence in luminous bacteria in low concentration. All inhibit at high concentration. All three retard the velocity of the luciferin-luciferase reaction—reversible action on the enzyme since the reaction rate over a wide range is independent of substrate concentration.

470. Shorb, M. S., Hansen, M. L. and Sowter, D. D., 1954, The effect of arsenicals on cecal bacteria from chicks. *Poultry Sci.* 33, 1080-1081.

Arsenicals stimulated growth of the bacteria.

471. Greaves, J. E., 1931, The influence of arsenic upon the biological transformation of nitrogen in soils. *Biochem. Bull.* 3, 2-16.

Several arsenic compounds stimulated nitrifiers and ammonifiers.

472. Hessayon, D. G., 1951, Double-action of trichothecin and its production in soil. *Nature* 168, 998-999.

Very small concentrations of trichothecin stimulated mycelial growth of *Fusarium oxysporum*. Higher concentrations were toxic.

473. Woolley, D. W., 1950, A study of the basis of selectivity of action of amino acid metabolites with analogues of pimelic acid. *J. Biol. Chem.* 183, 491-505.

As is seen in many other metabolites, this analogue of pimelic acid produced a growth stimulation when present in sub-inhibitory concentrations.

474. Beckwith, T. D. and Geary, E. M., 1940, Effect of indol-3-acetic acid on multiplication for *Esch. coli* and *E. typhosa*. *Journal of Infectious Disease* 66, 78-79.

In certain concentrations, indol-3-acetic acid, added to media with either *E. typhosa* or *E. coli*, stimulated growth. Higher conc. of this salt inhibited growth.

- 74a. Hanka, L. H. and Lockhart, W. R., 1958, Antimicrobial action as a basis for measurement of diethylstilbestrol. *J. Bacteriol.* **75**, 471-473.

They found a slight but characteristic stimulation of growth just below the threshold concentration of inhibition.

75. Southam, C. M. and Ehrlich, J., 1943, Effects of extract of western red-cedar heartwood on certain wood decaying fungi in culture. *Phytopathol.* **33**, 517-524.

Phenolic type compounds isolated from heartwood stimulated growth of otherwise sensitive organisms at certain concentrations. Suggest the term hormesis.

76. Waksman, S. A. and Woodruff, H. B., 1941, *Actinomyces antibioticus*. A new soil organism antagonistic to pathogenic and non-pathogenic bacteria. *Journal of Bacteriology* **43**, 241-249.

Although the authors describe as "bacteriostatic" the action of actinomycin, one photograph (p. 234) seems to show stimulation of growth of *Bac. mycoides* and *Sarcina lutea* around the edges of the bactericidal zone.

77. Hotchkiss, R. D., 1944, Gramicidin, tyrosidin and tyrothricin. *Adv. Enzymol.* **4**, 153-195.

Gramicidin stimulates oxidation in *Staph. aureus* while phosphorus uptake is inhibited.

- 77a. Moore, M., 1951, *In vivo* and *in vitro* effects of aureomycin hydrochloride on *Syringospora* (*Monilia*, *Candida*) *albicans*. *J. Lab. and Clin. Med.* **37**, 703.

78. Ungar, J. and Muggleton, P., 1946, The effect of penicillin on the growth of human type of *M. tuberculosis*. *Journal of Pathology and Bacteriology* **58**, 501-504.

Detailed description of technique concludes: pure penicillin has a stimulatory effect on growth rates of *M. tuberculosis*.

79. Pratt R. and Dufrenoy, J., 1947, Cytochemical mechanism of penicillin action. IV. Comparative responses of gram positive and gram negative bacteria. *J. Bact.* **54**, 719-730.

A zone of accelerated growth was seen external to the zone of inhibition.

80. Hobby, G. L. and Dawson, M. H., 1944, Effect of rate of growth of bacteria on action of penicillin. *Proc. Soc. Exptl. Biol. and Med.* **56**, 181-184.

Occasionally the bacterial count was higher in media containing 10-100 units of penicillin per tube than in control tube.

81. Eriksen, K. R., 1946, Studies on induced resistance to penicillin in staphylococci. *Acta. Path. Microbiol. Scand.* **23**, 284-292.

Growth of *S. aureus* is enhanced by sub-bacteriostatic concentration for penicillin.

82. Lipnik, M. J., Klignan, A. M. and Strauss, R., 1952, Antibiotics and fungus infections. *J. Invest. Dermat.* **18**, 247.

Growth stimulation of *Candida albincosis* by antibiotics.

83. Curran, H. R. and Evans, R. F., 1947, Stimulation of sporogenic and non-sporogenic bacteria by traces of penicillin or streptomycin. *Proc. Soc. Exper. Biol. and Med.* **64**, 231-233.

Low concentrations of penicillin or streptomycin stimulate the growth of many sporogenic and two non-sporogenic bacteria—concentration of these drugs efficacious as bactericidal or bacteriostatic agents for one species of microorganism, for another, perhaps coexistent infective agent, may be stimulatory.

484. Lampen, J. O., Morgan, E. R. and Slocum, A., 1957, Effect of nystatin on the utilization of substrates by yeast and other fungi. *J. Bacteriol.* **74**, 297–302.

Nystatin stops the growth of most fungi at 1–102/ml. It inhibits endogenous respiration, anaerobic and aerobic glucose utilization while lower levels stimulate both glucose oxidation and glycolysis.

485. Miller, W. S., Green, C. A. and Kitchen, H., 1945, Biphasic action of penicillin and certain other sulphonamide similarity. *Nature* **155**, 210–211.

Sulfonamides have a stimulation action and now certain concentration of penicillin in broth with *Staphylococcus aureus* caused increased growth (turbidity) above those tubes containing no antibiotic.

486. Huppert, M. and Cazin, J., Jr., 1955, Pathogenesis of *Candida albicans* infection following antibiotic therapy. II. Further studies of the effect of antibiotics on the *in vitro* growth of *Candida albicans*. *J. Bacteriol.* **70**, 435–439.

In broth cultures aureomycin, neomycin and bacitracin produce greater total growth than in control cultures. No stimulation was seen with magnamycin, erythromycin and aureomycin with parabens.

488. Welch, H., Price, C. W. and Randall, W. A., 1946, Increase in fatality rate of *E. typhosa* for white mice by streptomycin. *J. Am. Pharm. A.* **35**, 155–158.

At certain concentrations, streptomycin increased rather than decreased the fatality rate in mice infected with *E. typhosa*.

487. Stansfeld, J. M., Francis, A. E. and Stuart-Harris, C. H., 1944. Laboratory and clinical trial of patulin. *Lancet* **247**, 370.

Mice infected with *E. typhosa* had a higher death rate when treated with patulin than controls.

489. Rivièrè, C., Thely, M. and Gautron, G., 1947, Action acceleratrice exercée par certaines conditions, par la pénicilline sur l'évolution de la tuberculose expérimentale du cobaye. *C. R. Acad. Sci., Paris*, **224**, 1856.

Penicillin greatly accelerates death from tuberculosis in guinea pigs (hard to show since the drug is toxic to controls).

490. Randall, W. A., Price, C. W. and Welch, H., Demonstration of hormetic effect (increase in fatality rate) by penicillin. *Pub. Health* **37**, 421, 1947.

At certain dosage levels penicillin is capable of exerting a hormetic effect on the death of mice infected with *E. typhosa*. Levels of penicillin of 5–50 units appeared to stimulate the growth of the organism *in vitro* (as determined by immunity) which killed a greater proportion of mice than died in control groups.

491. Foley, G. E. and Winter, W. D., 1949, Increased mortality following penicillin therapy of chick embryos infected with *Candida albicans* var. *stellatoidea*. *J. Infect. Dis.* **85**, 268.

Penicillin increases the mortality of chick embryos inoculated with *Candida albicans*.

192. Seligmann, E., 1952, Virulence enhancing activities of aureomycin on *Candida albicans*. *Proc. Soc. Exptl. Biol. and Med.* **79**, 481-484.

Combination of non-pathogenic candida and non-toxic aureomycin solutions are toxic when injected at the same time into mice. The *in vivo* action lowered the resistance of mice.

193. Garrod, L. P., 1951, The reactions of bacteria to chemotherapeutic agents. *British Med. Journal* **1**, 205-210.

Extra food or cell exudates don't account for growth stimulation in this method: comparison of sizes of colonies on normal and on penicillin-containing media. Although growth stimulation was found in the presence of penicillin the author says three other variables are influential: age of culture, medium and temperature. He discusses the effect of each of these. He suggests the antibiotic stimulates growth of relatively dormant cells. Penicillin treatment may activate a quiescent tuberculous focus and bacteria appear where none were found before.

194. Hirsch, J. and Dosdogru, S., 1947, The antistaphylococcal effect of penicillin, streptomycin and 5,7-dichloro-8-hydroxyguinaldine. *Arch. Biochem.* **14**, 213-227.

Low levels of penicillin increased respiration in *S. aureus*.

195. Michael, J. G. and Brown, W., 1958, Relationships between bacterial resistance to serum and penicillin. *Proc. Soc. Exptl. Biol. and Med.* **97**, 104-107.

Prior exposure to low levels of penicillin produced phenotypic changes in resistance to serum in *E. coli*. Bacteria pre-grown in low levels of penicillin had significantly higher resistance to serum.

196. Smith, G. N., 1952, The influence of chloromycetin decomposition products on the growth of *Escherichia coli* and their effects on reversing the growth-inhibiting action of the antibiotic. *Arch. Biochem. and Biophys.* **40**, 314-322.

Bacterial decomposition products of chloramphenicol which stimulated the growth of *E. coli* are ethanolamine, p-nitrobenzaldehyde, 2-amino-1-(p-aminophenyl)-1, 3-propanediol, p-nitrophenylserine and 2-amino-1-(p-nitro-phenyl)-1,3-propanediol.

197. McElroy, W. D., 1947, The mechanism of inhibition of cellular activity by narcotics. *Quart. Rev., Biol.* **22**, 25-58.

A thoughtful review of the effect of different compounds on the stimulation of one system while inhibiting another.

198. Netien, G., Carraz, M. and Sotty, L., 1952, De l'action comparée de la streptomycine et de la dihydrostreptomycine au la croissance de tissus de carotte cultivés *in vitro*. *Compt. Rend. Soc. Biol. (Lyon)* **146**, 1339-1341.

Carrot tissue growth was stimulated by 10 ppm dihydrostreptomycin.

199. Barton, L. V. and MacNab, H., 1954, *Contr. Boyce-Thompson Res. Inst.* **17**, 419.

Seedlings grown in water had their growth stimulated by penicillin, oxytetracycline and thiolutin and wet roots were stimulated by polymyxins β and rimocidin.

200. Havinga, E., Lynch, V., Norris, L. and Calvin, M., 1953, The effect of certain biologically active substances upon photosynthesis and dark CO₂ fixation. *Recueil des Travaux Chimiques des Pays Bas* **72**, 597-611.

Here they draw a tentative conclusion that some antibiotics have a

positive metabolic function. Research was concentrated on aureomycin HCl and terramycin HCl and their effect on cell division of *Scenedesmus*. Algae growth was stimulated by aureomycin crystal outside of the zone of inhibition. The algae grew to a greater depth than in non-stimulated areas. Aureomycin increased the production for sucrose.

501. Maximov, N. A., 1930, A Testbook of Plant Physiology. McGraw Hill Book Co., page 308.

When applied in very weak doses, the majority of even the most poisonous substances will not depress, but stimulate growth. Phenol stops growth at 100 moles/100,000 liters but stimulates at 4-8 mole.

502. Stewart, J. and Smith, E. S., 1922, Some relations of arsenic to plant growth. Part 2. *Soil Sci.* **14**, 119-126.

Experiments of 1914 are reported. Low levels of disodium arsenate stimulated all plants tested (wheat, pea, radish, potato and bean). 2 ppm. Higher were general inhibitory; peas and wheat at 75 ppm.

503. Chapman, R. K. and Allen, T. C., 1948, Plant hormone effect of D.D.T. *J. Econ. Ent.* **41**, 616-623.

Stimulation and suppression of some vegetable plants by D.D.T.

D.D.T. stimulates growth (height) in plant roots and other parts. It also increases the number of blooms and gives about 20% weight increase. Above 0.0005-0.03% inhibition and deformities result.

504. Morrison, H. E., Mote, D. C. and Rasmussen, 1948, Effect of soil insecticides on plants. *J. Econ. Ent.* **41**, 374-378.

Increased vigor of cabbage and lettuce seedlings is seen in D.D.T. treated plants (430 ppm).

505. Wolfenbarger, D. O., 1948, Nutrition value of phosphatic insecticides. *J. Econ. Ent.* **41**, 818-819.

Parathion, HETP and TEPP increase the yield of potatoes independently of the insecticidal effect.

506. Heath, O. V. S. and Clark, J. E., 1956, Chelating agents as plant growth substances. *Nature* **178**, 600-601.

507. Brian, P. W., 1957, The effects of some microbial metabolic products on plant growth. Symp. Soc. Exptl. Biol. XI. The biological Action of Growth Substances. Academic Press, Inc., New York, pp. 166-182.

Indole acetic acid is produced by fungi, yeasts and bacteria. It suggests parasitic plant disease organisms produce indole acetic acid, gibberellic acid and other growth promoting substance faster than the host cell can remove it. This may explain where excessive cellular proliferation, such as crown gall, is seen in which more indole acetic acid is found in the cells. Corn smut, potato wart disease, apple canker and cedar-apple rust may be in this category. A lengthening of stem is seen in rice Bakanae disease produced by *Gibberella fujikurii* and other rusts, smut and downy mildew disease. Excessive branching may also be caused by increased auxin production in witches broom disease and leaf gall disease.

Gibberellin gives a three fold growth increase in dwarf varieties of pea and little reaction in tall varieties.

508. Cole, B. A., 1950, The effects *in vitro* of certain antibiotics on the growth of *Trichomonas foetus*. *Proc. Helminth. Soc. Washington* **17**, 65.

Bacitracin enhances the growth of protozoan, *Trichomonas factus*. It is suggested that this is a metabolite.

509. Firket, H. and Chevremont, M., 1954, Action de la pantetheine sur la croissance des cultures de tissus. *Compte rend. Soc. Biol.* **148**, 1689-1691.

Pantetheine stimulated the growth of chick embryo fibroblasts at a dilution of 1:15,000 while it was toxic at a dilution of 1:200.

510. De Armijo Valenzuela, M. and Wattenberg, J. M., 1954, Action of tetracycline, terramycin and aureomycin on tissue cultures. *Arch. Inst. Farmacol. Exptl. (Madrid)* **6**, 77-90. (*C. A.* **49**, 15070, 1955.)

At 100 ppm they all caused cell degeneration; at 50 ppm tetracycline and oxytetracycline have a growth stimulating action.

511. Hardin, A. and Young, W. J., 1911, The alcoholic ferment of yeast juice. Part VI. The influence of arsenates and arsenites on the fermentation of sugars by yeast juice. *Proc. Roy. Soc. Series B.* **83**, 451.

Arsenates and arsenites increase CO₂ production by yeast juice. Other compounds were not effective on enzymes directly (comes to enzymatic basis).

512. Smith, G. N., 1953, The possible modes of action of chloromycetin. *Bacterial Rev.* **19**, 19-29.

Action of chloromycetin on bacterial esterase is stimulatory in low levels.

513. Taliaferro, W. H. and Taliaferro, L. G., 1951, Effect of x-rays on immunity: a review. *J. Immunol.* **66**, 181-212.

Small amount of x-rays sometimes increases antibody formation and the immunity of animals to certain infections.

514. Gerhartz, H., 1909, Diphtheriegift und Röntgenstrahlen. *Berlin klin. Wchnschr.* **46**, 1800-1802.

Multiple irradiations partially protected rabbits against diphtheria toxins as measured by survival time.

515. Bisgard, J. D., Hunt, H. B. and Dickinson, R. H., 1944, Effect of x-ray irradiation upon bacterial toxemia in rabbits. *Radiol.*, **43**, 330-332.

516. Manoukhine, I. I., 1913, Sur la role des globules blancs et de la rate dans la production de l'alexine des hemolysines, des agglutinines et des bacterilysines. *Compt. rend. Soc. de Biol.* **74**, 1221-1222.

517. Dougherty, T. F. and White, A., 1946, Pituitary-adrenal cortical control of lymphocyte structure and function as revealed by experimental x-radiation. *Endocrinology* **39**, 370-385.

518. Kaznelson, P. and Lorant, J. S., 1921, Allgemeine Leistungsteigerung als Fernwirkung therapeutischer Röntgenbestrahlungen. *Münch. med. Wchnschr.* **68**, 132-135.

The agglutinin titer increases in patients irradiated locally several weeks after typhoid immunization.

519. Hektoen, L., 1920. Further observations on the effect of roentgenization and splenectomy on antibody production. *J. Infect. Dis.* **27**, 23-30.

520. Gager, C. S., 1936, The effect of radium rays on plants in Duggar, B. M., Biological Effects of Radiation, Vol. II. McGraw-Hill Book Company, Inc., New York, pp. 987-1013.

A review of the effect of radium rays on plants and plant tissue growth stimulation and germination, photosynthesis and respiration.

521. Johnson, E. L., 1936, Effects of x-rays upon green plants in Duggar, B. M. Biological Effects on Radiation, Vol. II. McGraw-Hill Book Company Inc., New York, 1936, pp. 961-983.

The summary indicates an injurious effect on green plants by x-rays in medium and heavy doses and a possible growth rate stimulation action of light doses.

522. Kersten, H. J., Miller, H. L. and Smith, G. F., 1943, Stimulative effects of x-rays on plants. *Plant Physiol.* **18**, 8-18.

Growth of corn is stimulated by low doses of x-rays.

523. Kimball, R. F., The effect of radiation on protozoa and the eggs of invertebrates other than insects. In Hollaender, A., Radiation Biology II. McGraw-Hill Book Company, New York, pp. 285-331.

Reviews activation of eggs by UV light, p. 30. Low level x-rays accelerate paramecium and cause increased permeability of *Barnea candida* eggs.

524. Barron, E. S. G., 1952, The effect of ionizing radiations on some systems of biological importance. In Symposium on Radiobiology, by J. J. Nickson. John Wiley and Sons, Inc., New York, pp. 216-239.

Cell respiration in *Corynebacterium creatinovorans* increases slightly when given 300-100 r of X-radiation; levels above that caused depression.

525. Smith, E. C., 1935, Effects of ultra-violet radiation and temperature on *Fusarium*. II. Stimulation. *Bull. Torrey Botan. Club* **62**, 151-164.

Very low doses of UV light stimulated vegetative growth (following a period of retarded growth) in *Fusarium* spores. More growth was seen from treated spores at 48 hours than in controls following a single exposure (best at 0.5 min.) to UV light. The response is different at different temperatures. It is better at 30°C than at 21°C or 25°C. Likewise 5 hours at 0°C or 45 min. at 7.5°C produced the same effect. She suggests anything which will cause a growth inhibition will give secondary stimulation.

526. Nadson, G. and Philippov, G., 1928, Action excitante des rayons ultra-violet sur le developement des levures et des moisissures. *Compt. Rend. Soc. Biol., Paris* **98**, 366-368.

Greater yeast growth around edges of the irradiated zone than outside the zone whereas the growth in the middle is retarded.

527. Chavarria, A. P. and Clark, J. H., 1924, The reaction of pathogenic fungi to ultraviolet light and the role played by pigment in this reaction. *Am. J. Hyg.* **4**, 639-649.

Short exposures to UV give growth stimulus in yeast while long exposures are lethal.

528. Hutchinson, A. H. and Newton, D., 1930, The specific effect of monochromatic light on the growth of yeast. *Can. J. Res.* **2**, 249-263.

Different wave lengths of light stimulated the growth of yeast best in poor media.

529. Kayser, E. and Delaval, H., 1925, Radioactive fixtures d'azote et levures alcooliques. *Compt. Rend. Acad. Sci. Paris* **181**, 151-153.

Small doses of radiation from radioactive sources stimulate fermentation.

530. Smith, E. C., 1936, The effects of radiation on fungi. In Duggar, B. M., *Biological Effects of Radiation*, Vol. II. McGraw-Hill Book Company, Inc., New York, pp. 889-918.

She reviews (p. 894) growth stimulation of fungi by UV radiation, increased cell size produced by UV interstices strong enough to inhibit cell division, the necessity of light for chlorophyll formation generally for spore formation in some fungi, stimulation of growth of sporangiophores and increased respiration. Small doses of x-rays give questionable stimulation (poor control of dose) and small doses of rays from radioactive substances are found to stimulate cell division; α -rays appear to stimulate more than β or γ rays.

531. Hinrichs, M. A., 1928, Ultraviolet radiation: stimulation and inhibition in lower organisms. *Proc. Soc. Exper. Biol. and Med.* 26, 175-177.

532. Kirschner, L. B., Prosser, C. L. and Quastler, H., 1949, Increased metabolic rate in rats after x-irradiation. *Proc. Soc. Exper. Biol. and Med.* 71, 463-467.

Mild x-ray treatment increases BMR in rats.

533. Lea, E. E., 1947, Increase in cell size. In *Action of Radiation on Living Cells*. Macmillan Co., New York, pp. 300-302.

Bacteria, yeast, protozoa, fungi, algae, bean root tip, mouse tumors and human tumor cells increase in size of cells or their nuclei following radiation. Some of this effect may be due to inhibition of division.

534. Russell, M. A. and Garner, J. M., Jr., 1947, Effects of neutrons on early root development of *Zea mays*. In *Neutron Effects on Animals*. Williams and Wilkins Company, Baltimore, pp. 58-65.

Small doses of fast neutrons have a stimulating effect on the emergence of lateral roots of corn. Growth of the primary root was at slightly faster rate in irradiated plants.

535. Lorenzy, E., Jacobson, L. O., Heston, W. E., Shimkin, M., Eschenbrenner, A. B., Deringer, M. K., Doniger, J. and Schwersthal, R., 1954.

Effects of long-continued total body gamma irradiation on mice, swine, pigs and rabbits. III. Effect on life span, weight, blood picture and carcinogenesis and the role of the intensity of radiation. In *Biological Effects of External X and Gamma Radiation*. Part I, by R. E. Zirkle. McGraw-Hill Book Company, Inc., New York, pp. 24-148.

Survival of longer life when 0.11 γ x-rays were administered daily in mice, p. 29, 30 and 32, and guinea pigs, p. 34. Weight of treated animals was greater than the controls and the incidence of lymphoid tumors decreased with low dosage and increased with higher dose of radiation.

- 535a. Cork, J. M., 1957, Gamma-radiation and longevity of the flour beetle. *Rad. Res.* 7, 551-557.

The life span of the flour beetle may be extended by a single relatively low dose (3000r) or chronic daily dose (100r) or γ -rays.

536. Townsend, C. O., 1897, The correlation of growth under the influence of injuries. *Ann. Bot.* 11, 509-532.

Single very slight injuries to corn and barley seedlings stimulated growth rate for one to several days. In more severe injury the growth acceleration is preceded by a period of retardation. A dilute continuous atmosphere of ether or a strong concentration of ether for a short duration will also accelerate growth of higher plants.

Removal of tree branches causes other branches and fruit to be more perfectly developed.

Disturbance of roots of garden plants during cultivation will cause more vigorous and rapid development of all their parts.

537. Crocker, W., 1948, *Growth of Plants*. Reinhold Pub. Co., New York, 1948.

Ethylene induces proliferation of plant tissues in low concentration. In higher concentrations it killed the plants: concentrations as high as 1/1 billion in air.

CO stimulates rooting in plant stems, as do ethylene, propylene and acetylene. Ethylene produces earlier flowers in pineapple. Ethylene gas produces growth stimulus in 20–120 min. (cell elongation), cell division increases in 48–72 hours, initiation of roots 5–10 days and formative effects in 4–10 days.

Chapter 6 lists many growth promoting "hormones."

Low temperature modifies the embryo to overcome dormancy and increase vigor and hasten the reproductive phase. Winter wheats need 8–10 weeks at low temp.: a flowering response in some plants is also brought about by low temperature.

538. Plough, H. H., 1942, Temperature and spontaneous mutation. *Biochemical Symposia* 6, 9–20.

539. Brauner, L. and Bunning, F., 1930, Geoelektrischer Effekt und Elektrotropismus. *Ber. Deut. Bot. Ges.* 48, 470–476.

Coleoptiles bend toward the positive pole and root tips bend toward the negative pole in a weak electric current. Cf. Chapt. 9, *Growth and Hormones in Plants*, by Boysen-Jensen, McGraw-Hill, 1936.

540. Rosene, H. F. and Lund, E. J., 1949, Bioelectric fields and correlation in plants. In W. E. Loomis, *Growth and Differentiation in Plants*. Iowa State College Press, Ames, Iowa.

236—"Weak electric currents may accelerate streaming; strong ones always retard it;"

541. Ruegamer, W. R., Bernstein, L. and Benjamin, J. D., 1954. Growth, food utilization and thyroid activity in the albino rat as a function of external handling. *Science* 120, 184–185, 1954.

Increased growth and improved food utilization were observed when rats were petted ten minutes each day. These rats had a decreased thyroid activity as compared to control unhandled rats.

542. Lillie, R. S., 1924, Reactivity of the cell. In *General Cytology*, by E. V. Cowdry. University Chicago Press, Chicago, pp. 165–234.

In all cases of reversible stimulation there is first some kind of disruptive or integrative or destructive process which is succeeded by a reconstructive process. The reversal is complete as shown by the fact that the system repeats the same cycle regularly for years on end. Plasma membranes undergo temporary breakdown or increased permeability during stimulation. The response is typically greater than the stimulus both in space and time; there is a transmission throughout the whole responding system. The stimulus thus propagates itself to involve more remote regions of the system. This involves transmission of the reactions by structural change, p. 202. "Variations of electrical potential accompany many all forms of response to stimulation, apparently without exception."

" . . . they are associated with variations of permeability and sensitivity, and are characteristically reversible."

543. Luckey, T. D., 1956, Mode of action: First International Conference on Antibiotics in Agriculture, National Academy of Sciences and National Research Council, p. 162.

A drug in small quantities stimulates the cell to prepare it for stress. The stimulus may present such a small thing that the cell overcompensates by making too many enzymes, etc.

544. Lamanna, C. and Mallette, M. F., 1953, Basic Bacteriology and Its Biological and Chemical Background. Williams and Wilkins Co., Baltimore, pp. 598-601.

The mechanisms for improved performance of bacteria under the influence of a stimulus may be a permeability effect from absorption on the cell surface of the poison. The toxin may inhibit one reaction which will allow another to proceed at a faster rate, or it may combine with the enzyme to change its characteristics without inactivating it to give an effective increase in enzymatic action.

545. Smith, G. N., 1952, The influence of chloromycetin decomposition products on the growth of *Escherichia coli* and their effects on reversing the growth inhibiting action of the antibiotics. *Arch. Biochem. and Biophys.* **40**, 314-322.

CHAPTER IV

THE INFLUENCE OF ANTIBIOTICS ON PLANTS AND PLANT DISEASE CONTROL

BY R. N. GOODMAN

A. INTRODUCTION

The impact of the antibiotic era upon plant culture has been primarily in the area of disease control. However, these compounds have also elicited morphological effects upon plants, with both growth inhibition and stimulation being observed. In addition, subtle physiological changes and genetic modifications have also been reported.

Antibiotics have made more practicable the internal protection of a plant against invading pathogens, as well as the eradication of established infections. The systemicity of streptomycin, the tetracyclines, cycloheximide and griseofulvin in some plant tissues has been firmly established.

Successful therapy and prophylaxis against plant diseases have been for the most part, against the bacterial pathogens. Furthermore, many of these phyto-bacterial diseases had no successful control measure, prior to the advent of antibiotics. For these diseases, streptomycin has been the antibiotic of choice.

The most prominent antifungal antibiotic has been cycloheximide. Although extremely effective against some pathogens, often in concentration lower than 1 mcg/ml (*in vivo*), its spectrum is quite narrow. Griseofulvin is another antifungal antibiotic which appears to be gaining commercial acceptance.

A wide range of host responses to antibiotics have been observed. For example, antibiotics have been reported to affect such plant processes as photosynthesis, pigmentation, polyphenolase activity, organic acid synthesis, chromosomal behavior, etc. The magnitude of the host response displayed seems to be dependent upon the plant species sensitivity, the specific antibiotic and concentration used.

Transport of large organic molecules in plants is still a relatively unexplored scientific frontier. Therefore, the movement of antibiotics in the xylem and phloem elements of the vascular system has received particular attention. It is apparent from some of these studies that antibiotics might well be used as "biological tracers." The precise

methods available for detecting these compounds in small quantities make them unusually fine tools with which to study absorption and translocation of organic substances in plants.

A number of excellent reviews have been published on the efficacy of antibiotics as chemical agents for plant disease control.^{13,105,187,206a,206b,222,297,367} An extensive review of this type of the accomplishments up to 1956 was prepared by Zaumeyer.³⁷¹

Reviews on specific types of antibiotic effects have also been published. Thus, Anderson and Nienow¹² have described some phytotoxic effects of streptomycin, and Crowdy and Pramer⁸¹ reviewed the absorption and translocation data available in 1955. Recently, an excellent recapitulation of the available data on such effects of antibiotics on plants as inhibition, stimulation, phytotoxicity and translocation was published by Brian.⁵⁰

The aim of this chapter is to report the many significant studies of the effects of antibiotics on higher plants, to present them systematically and to comment upon their implications.

B. METHODS OF APPLICATION

From a review of the literature it would appear that almost every conceivable method of administering antibiotics to plants has been exploited.

These methods have varied with the nature of the disease and the characteristics of the plant. Factors such as the type of disease (vascular wilt, leaf spot, etc.), stage of development of the plant, stage of development of the disease, size of the plant, location of the plant (field or greenhouse), and accessibility of the planting have all affected the manner of antibiotic application. (See Table 4-1.)

Sprays, dusts, dips, and soil treatments have been used predominantly. However, several other unique methods of administration have also been employed.)

1. SPRAYS

The foliar spray is used regularly and as a matter of routine by plantsmen throughout the growing season to suppress insects and diseases. The spray has been found admirably suited for administering antibiotics to control some diseases of fruits, vegetables, and agronomic crops. It is the preferred method for applying streptomycin to control *Erwinia amylovora*, which causes fireblight of apple and pear;^{133,136,183,259,357,359} *Xanthomonas juglandis*, which causes walnut blight;^{16,19,24} *X. vesicatoria*, which causes bacterial spot of tomato and pepper;^{65,68,72} and *Pseudomonas*

TABLE 4-1
STUDIES ON THE CONTROL OF PLANT BACTERIAL AND FUNGAL PATHOGENS

Disease (Bacterial)	Antibiotics used*	Antibiotic of choice	Type of application	Field studies	Greenhouse, lab. studies
<i>Agrobacterium tumefaciens</i> Crown gall	st, ox, ch, pe, sr, pa, as, co, cl, ty, po	te	dip, injection	206	29, 36, 53, 54, 56, 96, 97, 169
<i>Bacterium armeniacae</i> Apricot blight	st, gz, **		spray, dip		248, 250
<i>Bacterium stewartii</i> Bacterial wilt of sweet corn	st, ox, cy, pe		spray, seed	231	304, 355
<i>Corynebacterium michiganense</i> Tomato canker	st, pe, ch, gz, gl	st	seed	17, 251	22
<i>Corynebacterium sepedonicum</i> Potato ring rot	st, ba, pe		dip	233	335
<i>Erwinia</i> sp. Philodendron leaf rot	st		spray		244
<i>Erwinia amylovora</i> Fireblight	ne, ox, ch, st, th, pe	st	dip, dust, spray, capsule—19, 24	18, 20, 21, 24, 25, 61, 106, 133, 134, 138, 140, 183, 201, 202, 245, 247, 259, 310, 357, 358, 359	3, 5, 6, 23, 135, 136, 137, 174
<i>Erwinia atrospetlica</i> Potato black leg	st, ds, pe, ba, ri, th	st	dip	41, 116	39, 40, 42
<i>Erwinia caratovora</i> Bacterial soft rot	st, ox, ch		spray, dip	51, 320	
<i>Erwinia carnegiana</i> Cactus blight	pe		injection	43, 52	
<i>Erwinia chrysanthemi</i> Bacterial wilt of chrysanthemum	ox, ch, st, ne	st	dip		307

TABLE 4-1—(Continued)

Disease (Bacterial)	Antibiotics used*	Antibiotic of choice	Type of application	Field studies	Greenhouse, lab. studies
<i>Erwinia tracheiphila</i> Bacterial wilt of cucumbers	st, ox, ne		spray	354	
<i>Pseudomonas apii</i> Bacterial blight of celery	st	st		69	
<i>Pseudomonas caryophylli</i> Carnation wilt	st, ox		dip		127
<i>Pseudomonas fluorescens</i> Potato seed piece decay	st, ds, pe, ba, ri, th	st	dip	41	39, 40
<i>Pseudomonas glycinea</i> Soy bean bacterial blight	st, ox, ch, ma, ne ma, ne	st	spray	198	
<i>Pseudomonas lachrymans</i> Angular leaf spot of cucumbers	st	st	seed	17, 100	22
<i>Pseudomonas mors-prunorum</i> Bacterial canker of cherry	st	st	spray	79	
<i>Pseudomonas phaseolicola</i> Halo blight	st	st	seed, spray	184, 361, 366	261
<i>Pseudomonas syringae</i> Stone fruit blast	st, ds, ox, ch, po, th	st	spray	58, 109	108, 110, 114
<i>Pseudomonas tabaci</i> Tobacco wildfire	st	st	spray	31, 172, 173, 203, 302, 314, 315	
<i>Xanthomonas campestris</i> Cabbage black rot	co		seed		326
<i>Xanthomonas malvacearum</i> Angular leaf spot of cotton	st		seed	248	22
<i>Xanthomonas pelargoni</i> Geranium rot	st	st	drench		372
<i>Xanthomonas phaseoli</i> Common blight	st, al	st	spray, seed	11, 184, 237, 252, 253, 254	154, 155, 160

TABLE 4-1—(Continued)

Disease (Bacterial and Fungus)	Antibiotics used*	Antibiotic of choice	Type of application	Field studies	Greenhouse, lab. studies
<i>Xanthomonas pruni</i> Bacterial spot of peach	st, ne	st	infusion, spray	86, 87, 103, 104, 141, 310, 319	55
<i>Xanthomonas vesicatoria</i> Bacterial spot of tomato and pepper	st, ox	st		7, 62, 65, 66, 68, 70, 71, 72, 75, 77, 211	210
Disease (Fungus)					
<i>Alternaria solani</i> Early blight of tomato	gr		drench, spray		46, 89
<i>Ascochyta pisi</i> Pea blight	ri, pi, cy		seed		94, 95, 275, 345
<i>Botrytis cinerea</i> Storage rot	gr, cy	gr	drench, spray	307	46, 303, 331
<i>Bremia lactucae</i> Downy mildew of lettuce	st			73	
<i>Ceratocystis fagacearum</i> Oak wilt	cy		spray	121	
<i>Cladosporium cucumerinum</i> Cucumber scab	cy		spray	98	
<i>Cocomyces hiemalis</i> Cherry leaf spot	cy	cy	spray	60, 130, 242, 243, 278, 334	167, 168
<i>Cronartium ribicola</i> White pine blister rust	cy		paint	257	
<i>Curvularia lunata</i> "Fading out" of turf	cy	cy	spray	190	
<i>Dactylium dendroides</i> Mushroom mildew	gr, an, ol, cy, ri		drench, aerosol	142, 324	
<i>Erysiphe graminis</i> var. <i>tritici</i> Powdery mildew of wheat	cy, an		spray		76, 282

TABLE 4-1—(Continued)

Disease (Fungus)	Antibiotics used*	Antibiotic of choice	Type of application	Field studies	Greenhouse, lab. studies
<i>Erysiphe polygoni</i> Powdery mildew of hop clover	cy		spray		120
<i>Fusarium oxysporum</i> var. <i>lycopersici</i> Tomato wilt	th				147
<i>Graphium ulmi</i> Dutch elm disease	cy		spray	186,330	
<i>Gymnosporangium juniperis virginianae</i> Cedar-apple rust	cy		spray	325,327	
<i>Helminthosporium</i> sp. Melting out	cy	cy	spray	337,338	
<i>Helminthosporium victoriae</i> Victoria Oak Blight	hB,my		seed		220,221,297
<i>Monilinia fruticicola</i> Peach brown rot	pa,cy,my,fu,ol		spray, dip	182,274,278,328,364	2,4,331
<i>Peronospora destructor</i> Onion mildew	cy		dust	267	
<i>Peronospora parasitica</i> Downy mildew of broccoli	cy,an,my,st,en, fm,ne,th,fi,ti,te	st	spray	262,263	265
<i>Peronospora tabacina</i> Tobacco blue mold	st	st	spray	14,163	
<i>Phytophthora infestans</i> Late blight of potato, tomato	st,ri,ox,pe,ds,po, ba,th,ch,co	st	dip spray, drench	258	38,372
<i>Phytophthora phascoli</i> Downy mildew of lima beans	st	st	spray	78	368,370
<i>Pyricularia oryzae</i> Rice blast	bl		spray		125
<i>Podosphaera leucotricha</i> Apple mildew	cy		spray	206b	

TABLE 4-1—(Continued)

Disease (Fungus)	Antibiotics used*	Antibiotic of choice	Type of application	Field studies	Greenhouse, lab. studies
<i>Plasmodiophora brassicae</i> Cabbage club root	gr		drench		305
<i>Podosphaera leucotricha</i> Apple mildew	cy		spray	33	
<i>Pseudoperonospora cubensis</i> Downy mildew of cucumber	st,en,gm,pa,ox, my,po	st	spray	63	27,30
<i>Pseudoperonospora humuli</i> Downy mildew of hops	gr,st	st	spray		188
<i>Puccinia graminis</i> Bluegrass rust	hB,pa,cy,st,ds,ox, pl,ty,ne,sr,th,ba, gt,en		spray	67,346	165,166,318
<i>Puccinia menthae</i> Mint rust	cy		dust	266	
<i>Puccinia rubigo-vera</i> Wheat stem rust	cy		air, spray	229	
<i>Pythium</i> sp. Damping off	fi,cy		seed, drench		128,162
<i>Pythium aphanidermatum</i> Cottony blight of rye grass	cy		spray	351	
<i>Rhizoctonia solani</i> Root rot	cy		spray, drench	90	311
<i>Sphacelotheca sorghi</i> Sorghum smut	cy,F-17		seed, slurry	279	226,227
<i>Sphaerotheca humuli</i> Powdery mildew of raspberry	cy		spray	123,365	

TABLE 4-1—(Continued)

Disease (Fungus)	Antibiotics used*	Antibiotic of choice	Type of application	Field studies	Greenhouse, lab. studies
<i>Sphaerotheca pannosa</i> var. <i>rosae</i>	an,gr,cy	cy	spray	204,327	9
Powdery mildew of roses	fi		seed		177,178
<i>Stemphylium solani</i>	cy		seed, dust		
Tomato gray leaf spot	cy		spray	274	
<i>Tilletia</i> sp.	pa,F-17,ol,an,fu,gr,fi		spray		2,292,293,294,369,373
Covered smut of wheat	cy		seed		176
<i>Uncinula necator</i>	st		seed	277	
Powdery mildew of grapes	fi		seed		9,213,214,215,216
<i>Uromyces phaseoli</i>					
Bean rust					
<i>Ustilago kollerii</i>					
Covered smut of oats					
<i>Ustilago nigra</i>					
Loose smut of barley					
<i>Venturia inaequalis</i>					
Apple scab					

* Albamycin	al	Dihydrostreptomycin	ds	Grizemin	gz	Pleocidin	pl
Anisomycin	an	Endomycin	en	Helixin B	hB	Polymyxin	po
Aspergillie acid	as	F-17	F-17	Magnamycin	ma	Rimocidin	ri
Ayactin	ay	Filipin	fi	Mycostatin	my	Streptomycin	st
Bacitracin	ba	Fungichromin	fm	Neomycin	ne	Streptothricin	sr
Blasticidin	bl	Fungicidin	fu	Oligomycin	ol	Tetracycline	te
Candidin	ca	Gliotoxin	gt	Oxytetracycline	ox	Thiactin	ti
Chloramphenicol	co	Globisporin	gl	Patulin	pa	Thiolutin	th
Chlortetracycline	ch	Gramicidin	gm	Penicillin	pe	Tyrosin	ty
Cycloheximide	cy	Griseofulvin	gr	Pimaricin	pi		

** Russian antibiotics, 6, 15, 15n, 18

tabaci, which causes tobacco wildfire.^{172,173,278} Cycloheximide sprays have been reported extremely effective in eradicating established infections of cherry leaf spot, caused by *Coccomyces hiemalis*,^{60,120,167}

The relatively low levels of antibiotic that can be introduced into plant tissue through foliar sprays appears to be a major objection to this method of application. It is perhaps for this reason that spray applications are primarily protective rather than eradivative.

2. DUSTS

Occasionally conditions favor the application of disease inhibiting substances as dusts and streptomycin dust formulations have been reported successful. As a rule however, results by Ark,²⁴ Heggstad *et al.*,¹⁷³ and Goodman¹³⁹ have demonstrated sprays to be superior to the dust forms (see Table 4-2).

TABLE 4-2
EFFECTIVENESS OF STREPTOMYCIN SPRAYS AND DUSTS IN
CONTROLLING FIREBLIGHT (*ERWINIA AMYLOVORA*) ON
JONATHAN APPLE (After Goodman 139)

Treatment	No. of trees	Total blight count	Blight/tree
Block I			
Checks	9	214	24
Sprayed	13	73	6
Dusted	12	167	14
Block II			
Checks	7	967	138
Sprayed	10	119	12
Dusted	9	718	80

3. DIPS

The immersion or dipping of whole plants or plant parts into pesticides is a common agricultural practice. It is particularly well suited to the application of antibiotics, for a number of reasons. First, large numbers of plants can be treated with relatively small quantities of these expensive materials. Second, excellent contact between plant and antibiotic can be effected, and finally, the exposure time, that is frequently critical, can be perfectly controlled.

Experimentally and practically, antibiotics have been shown to be effective as dips, and the accumulated evidence may be conveniently summarized according to the plant part treated.

a. Seed Treatments

Antibiotics have effectively decontaminated seed surfaces, and reduced thereby the incidence of disease when the seeds were subsequently planted.^{128,176,177,178}

There is also exceptionally good evidence which suggests that some antibiotics are effective systemically and that they eradicate internal seed infections.^{17,22,94,275}

b. Vegetative Tissue (Stems, Tubers, etc.)

One of the earliest attempts to eradicate a phytopathogen was that of Brown and Heep,⁵⁵ who immersed plum bud sticks (stems) infected with *X. pruni* into a streptomycin solution in an enclosed chamber which was subsequently placed under negative pressure. A similar experiment was conducted by Mirzabekyan;²⁵⁰ and Alcorn and Ark³ dipped pyracantha cuttings into streptomycin and the tetracycline antibiotics to protect them against artificial inoculations with *E. amylovora*. Analogous experiments were conducted with chrysanthemum,³⁰⁷ and carnation cuttings.⁵

It has also been reported that immersion of stems of a number of plant species infected with crown gall, *Agrobacterium tumefaciens*, in a number of antibiotic solutions suppressed gall development, and in some instances eradicated the infection.^{29,36,93,169}

Finally, potato tubers, which are actually stem tissue, are now, as a routine procedure, dipped in streptomycin at 100 mcg/ml to control black leg due to *E. atroseptica*, and to reduce seed piece decay from *P. fluorescens*.^{39,40}

c. Post Harvest Dips of Fruit and Foliage

This practice is described more completely elsewhere in this book. Streptomycin and the tetracyclines have been employed in post harvest treatments to suppress soft-rot development caused by *E. caratovora*, and a number of antifungal antibiotics, actidione, mycostatin, filipin, have been used to limit the development of *M. fructicola*, *Botrytis*, and *Rhizopus* rots.^{4,74,99,182,320}

4. THE INJECTION TECHNIQUE

This method has rarely been successful in administering chemicals to plants, since it is difficult to attain with it a uniform distribution throughout the plant. Nevertheless, the injection method was employed in what appears to be the first *in vivo* experiment with antibiotics. Brown and

Boyle⁵³ sought to eradicate crown gall infections caused by *A. tumefaciens*, in *Bryophyllum*, by injecting the plants just below the galls. The treatment resulted in a localized suppression of growth and an apparent accentuation of the irregularities on the galls' surface. These effects suggested an incomplete lateral diffusion from the point of administration. Seeking to improve their method of application, Brown and Boyle wrapped the galls in cotton impregnated with penicillin, but achieved only inactivation of the cells at the surface of the gall. However, penetration of the gall by the antibiotic was attained by puncturing the cotton-swathed gall with a needle several times. Galls so treated were completely destroyed. Later, Boyle⁴³ injected one quart of penicillin into a giant cactus with limited success in controlling bacterial necrosis caused by *E. carnegiana*.

Dunegan^{103,104} using the "plasma" method, or gravity feed system, was able to infuse peach trees with 28.4 liters of water containing 1.7 grams of oxytetracycline. This infusion appeared to reduce the severity of *X. pruni* infection (bacterial shot-hole). In subsequent experiments, Dunegan and coworkers found that oxytetracycline solutions were not as readily absorbed as the water controls. For example, only 7–10 liters of a 100 mcg/ml oxytetracycline solution were absorbed, whereas control trees absorbed 40 liters of water. Oxytetracycline also reduced the rate of absorption. Ark,²⁰ Dunegan,¹⁰³ and Goodman¹⁴³ have all administered streptomycin to fruit trees in capsules inserted into the tree trunks. Goodman observed that capsules and contents were dissolved and absorbed within two weeks after placement.

A rather unique experimental technique for infusing streptomycin into specific areas of plant tissue was described by Dunegan and Wilson.¹⁰⁷ They used a thread wick suspended at one end in a vial containing the antibiotic, with its other end inserted into a puncture of leaf or stem tissue.

5. SOIL AND SAND APPLICATIONS

Perhaps the most logical and direct method of applying antibiotics to plants is through their root systems. There are, however, two extremely important barriers to the use of this method. First, these organic compounds are rapidly metabolized by the soil microflora; and second, many of them are inactivated by adsorption by colloids in the soil.

In a series of exhaustive studies, Gottlieb and his students^{241,317} demonstrated that the highly basic antibiotics such as streptomycin, streptothricin, and neomycin, and the polypeptide antibiotics, such as circulin, rimocidin, subclin, and actinomycin, are inactivated in the presence of soil. Their data also suggest that the amphoteric tetracyclines, in either

alkaline or acid forms, are adsorbed by both illite and montmorillonite clays.

One might suspect, therefore, that the neutral antibiotics might be more readily absorbed from soil through plant roots. This reasoning appears to be sound, since Brian and co-workers⁴⁶ recovered griseofulvin from guttation water of oat plants that had received a soil drench containing 50 mcg/ml of the antibiotic. Similarly Rich³⁰⁵ recorded the effectiveness of griseofulvin at concentrations 20 and 40 mcg/kg of dry soil in suppressing *Plasmodiophora brassicae*. Gottlieb *et al.*¹⁵¹ observed also that cycloheximide, which is weakly acid, and clavacin, which is neutral, were not removed from aqueous solution by soil. Consequently, both antibiotics inhibited sensitive organisms when applied to the soil. The findings of Gregory *et al.*,¹⁶² that actidione at concentrations of 6.2 and 25.0 mcg/ml in the soil completely controlled damping off (*Pythium debaryanum*) of alfalfa, tend to suggest also that this antibiotic is not seriously inactivated by the exchange phenomenon. Finally, one might note the experiments of Gopalkrishnan and Jump¹⁴⁷ with the neutral antibiotic thiolutin. Their data suggest that *Fusarium* wilt of tomato was not controlled when this neutral antibiotic was added to sand or soil. Excellent results, however, were obtained when tomatoes were grown in solutions containing the antibiotic. This inconsistency may be more apparent than real and is considered again in the section of this chapter devoted to mode of action.

6. OTHER METHODS OF APPLICATION

In studying the effect of streptomycin on *A. tumefaciens*, De Ropp⁹⁷ infused carrot cylinders with the antibiotic by placing them on agar containing it. Moss²²³ combined cycloheximide at concentrations of 150 mcg/ml with an isoparaffinic base oil to make a paint with which cankers of the Western white pine blister rust *Cronartium ribicola* were treated. A streptomycin-lanolin paste has been used successfully by the writer in treating extensive fireblight trunk cankers. The lanolin preparation flows easily upon mild heating and is applied with a brush to the peripheries of the cankers after the diseased tissue has been removed.¹³⁹ Skiver³¹⁸ introduced antibiotics into a mist-propagation system in an attempt to control blight and rust of carnations caused by *Alternaria dianthi* and *Uromyces caryophyllinus*. In order to apply antibiotics to small grain plantings which, under field conditions, are grown in large acreages, Livingstone resorted to aerial applications. He used cycloheximide to control stem rust of wheat caused by *Puccinia rubigo-vera*.²²⁹ The inaccessibility of mushroom plantings to conventional spraying and

dusting procedures led the writer to apply cycloheximide as an aerosol in efforts to control mushroom mildew caused by *Dactylium dendroides*.¹⁴²

C. STABILITY AND PERSISTENCE OF ANTIBIOTICS

Once an antibiotic has been applied to a plant, plant part, or to the atmosphere in which the plant is growing, it usually has but a limited time to exert its activity against the pathogen. This period of activity or persistence varies directly with the stability of the compound. Stability is in turn influenced by a series of degradation and physical deactivation reactions. Further, these reactions occur continuously, both externally (in the microenvironment) and internally (within the plant).

1. EXTERNAL REACTIONS

The fate of some antibiotics in the soil has already been discussed, and it is readily apparent that those of cationic and amphoteric nature remain active for comparatively short periods of time. The experiments of Siminoff and Gottlieb³¹⁷ offer the opportunity of a closer look at the reactions involved in the deactivation of streptomycin by the clay colloids in soil. The authors described streptomycin as a trivalent cation and reacted it with Wyoming bentonite (Montmorillonite) and Illite types of clay. Both clays have 2:1 crystal lattice structures. However, the bentonite lattices have the capacity to expand, thus presenting additional adsorbing surface, whereas the Illite lattices are fixed (see Figs. 4-1 and 4-2). It is understandable, therefore, that the bentonite was found to adsorb 80 units of streptomycin and Illite only 14.5 units per milligram of clay.

Attempts to remove streptomycin from the bentonite clay by exchange with hydrogen (dialysis) were unsuccessful; and only 5% of the amount adsorbed could be recovered through exchange reactions with large organic dye molecules, such as methylene blue and janus green. It is likely, therefore, that the remainder was trapped between the expanding alumino-silicate sheets, and only those molecules at exposed surfaces could be removed. It might be added that in addition to the electrical forces attracting large positively-charged organic molecules, such as streptomycin, Hendricks¹⁷⁵ has shown that powerful van der Waals forces also come into play. These forces produce molecular arrangements between the sheets that make many of the exchange spots inaccessible to exchange reactions. Thus, the basis for the reports by Ark and co-workers^{21,24,26} that the bentonite clays are unsuited as diluents for dust formulations of streptomycin is now quite clear. It should be equally apparent why they found pyrophyllite (Illite) clays to be better suited

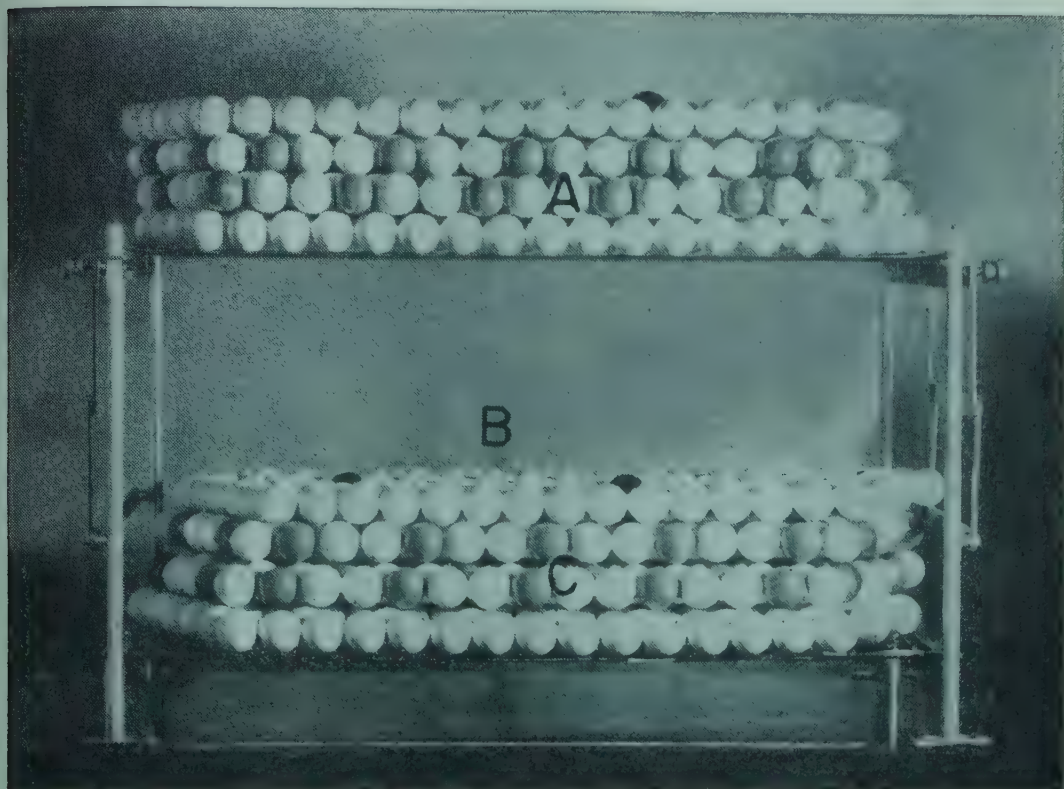


FIGURE 4-1. MODEL OF AN EXPANDING LATTICE (MONTMORILLONITE) CLAY. *Courtesy of C. E. Marshall.* A and C—Expanding aluminosilicate sheets. Outer surfaces of these sheets are negatively charged and are available to positively-charged streptomycin molecules. B—Space between sheets is available to streptomycin when hydrated.

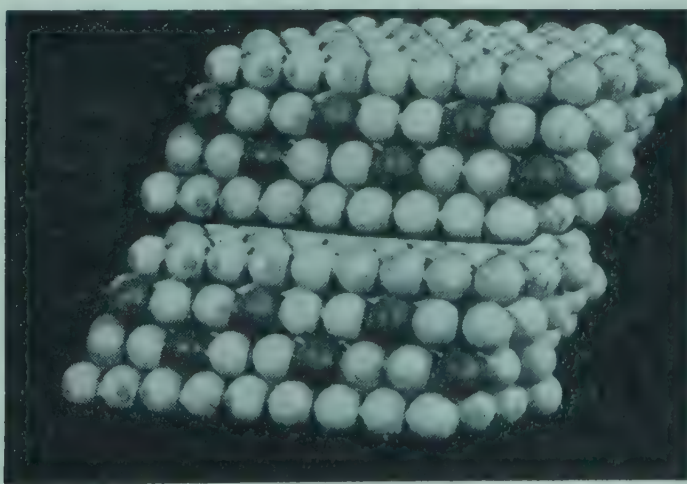


FIGURE 4-2. MODEL OF AN ILLITE NON-EXPANDING LATTICE CLAY. *Courtesy of C. E. Marshall.* Non-expanding aluminosilicate sheets. Adsorption possible only at outer surfaces.

for this purpose. In fact, Ark²⁸ has reported that expressate from the primary leaves of bean plants treated with a 1,000-ppm streptomycin-pyrophyllite dust contained as much as 6 mcg/ml of the antibiotic three days after treatment.

Soil reaction, or pH, which is actually a physico-chemical characteristic of soil, also seems to influence the persistence of antibiotics in soils. Gregory *et al*¹⁶¹ offer as evidence their findings that clavacin, a neutral antibiotic, remained active for 1 day in alkaline soils and for 2-4 days in acid soils. The weakly-acid cycloheximide (actidione) was detected for 8 days in alkaline soils, and for 14 days in acid soils. Similar data concerning actidione have been reported by Rushdi and Jeffers.³¹¹ According to Gregory *et al*,¹⁶¹ fradycin, a weakly-basic antibiotic, was strongly adsorbed by acid soils, but 90% of this compound remained in both acid and alkaline soils after 14 days. Hessayon¹⁸¹ reported an analogous situation for trichothecin, which was found to be strongly adsorbed by the soil, but appeared to resist biological degradation. On the other hand, gliotoxin, which is basic, seemed to be extremely labile in soil, being undetectable a few hours after application.¹⁶¹

The fact that biological degradations of antibiotics occur in the soil has been directly established by Pramer and Starkey.²⁸³ They have described the decomposition of streptomycin in soil and have ascribed its destruction to a Pseudomonad. Additional evidence in this respect was forthcoming from the data of Katz and Pienta¹⁹⁶ who seem to be quite sure that the decomposition of actinomycin is accomplished by an enzyme of a bacterium of the genus *Achromobacter*. The authors state that non-specific adsorption of the antibiotic by cells of the bacterium does not appear to be involved.

Additional external reactions strongly influence the stability of antibiotics. For example, Ammann⁹ showed that filipin, an antifungal antibiotic produced by *Streptomyces filipinensis*, was inactivated by light. This is a reaction common to other polyene antibiotics. He also demonstrated that the activity of filipin in apple foliage was reduced to 15% after 1 day, and to 1% after 3 days. Leben and Keitt²¹⁶ studied the stability of antimycin on apple leaves. They found that some activity was lost after 96 hours and that the antibiotic had a half-life of four days. They further noted that antimycin retention on leaf surfaces following precipitation was poor. Seventy-five percent was lost from the first 0.25 inches, and less than 10% remained after 1.0 inches had fallen.

The protracted stability of streptomycin is indicated by Dye¹¹² who states that once this antibiotic has been absorbed by the leaf, it is not washed off by precipitation, nor is it destroyed by the actinic rays of the sun. Further evidence regarding the stability of this compound was

presented by Goodman *et al.*¹⁴⁶ They reported that spinach treated with streptomycin contained antibiotic activity following a 3-minute exposure to live steam and a 3-minute rinse in a cold water spray. The heat stability of streptomycin has also been reported by Brody and Francis.⁵¹ Miric and Dunegan²⁴⁸ have reported the elucidation of an antibiotic substance produced by *Aspergillus niger*, which was extremely thermostable, in addition to being active against *X. pruni*.

2. INTERNAL REACTIONS

The fate of antibiotics after they are within plant tissue has scarcely been studied. From clinical investigations it is known that streptomycin is inactivated by hydrogen sulfide, cysteine, hydroxylamine, and certain sulfhydryl compounds. Timonin³³³ also found patulin (clavacin) to be inactivated by compounds containing —SH groups. Evidence suggesting internal chemical degradation of an antibiotic appears in data presented by Prescott and co-workers^{290,291} who reported that cycloheximide applied to ripe cherries, still on the tree, had a half-life of 24 hours. This rate of inactivation was much more rapid than that experienced with aqueous solutions of similar pH and temperature, and appeared to be an enzyme-fostered degradation. This rapid degradation of cycloheximide was not encountered by Wallen and Millar³⁴⁸ who applied the antibiotic as a spray to leaves of wheat plants. As a result of a single application, they could recover the antibiotic for at least 5 weeks.

The literature abounds with reports of the persistence of streptomycin in plant tissue, and there are a number of investigations which suggest that the tetracyclines are almost as stable. Morgan *et al.*²⁵⁵ detected residues of streptomycin in apple leaf tissue for at least 27 days after a single spray application of 100 mcg/ml. Experiments by Robinson and co-workers³⁰⁷ revealed that chrysanthemum cuttings dipped for 24 hours in 1,000 mcg/ml of either streptomycin or oxytetracycline still evinced antibiotic activity 8 weeks after treatment. It was noted, however, that streptomycin persisted longer than did oxytetracycline. A similar observation was made by Alcorn and Ark⁶ who found streptomycin to persist longer than tetracycline in carnation cuttings. Goodman and Johnston¹⁴³ placed streptomycin in capsular form into bore-holes of trunks of 10-year-old apple trees and dipped potatoes for 1 minute into streptomycin solutions. They detected antibiotic activity in the apple fruit and foliage for more than 100 days, and in the potatoes for four months. Applications of streptomycin to peach roots and stems by Chamberlin⁵⁸ permitted recovery of the compound in shoot apices after 5 months. The all-time record for streptomycin stability in plant tissue

is probably held by the experiment of Ark,²⁵ who placed 1.1 grams of streptomycin in the trunk of a Winter Nellis pear tree. The antibiotic was applied in March of 1954, and evidence of its presence did not become apparent during that growing season. The following year, in late March, characteristic streptomycin phytotoxicity symptoms were visible, and bioassays showed antibiotic activity in both fruit and foliage.

A long period of stability was also reported for chlortetracycline by Sutton and Bell³²⁶ who dipped swede seed into 1,000 mcg/ml solutions of the antibiotic and were able to detect activity in the dried seeds 9 months after treatment. Klemmer and co-workers,²⁰⁶ in their classic studies of crown gall (*A. tumefaciens*) inhibition with antibiotics, were able to detect chloramphenicol as well as oxytetracycline in tomato tissue for at least 10 days.

Crowdy⁸⁵ has calculated that the half-life of griseofulvin in broad beans is 4 days, whereas Brian *et al*⁴⁶ have been able to demonstrate that cut shoots of several plant species immersed in 10–100 mcg/ml of griseofulvin contained this antibiotic in their upper leaves after 214 days.

D. COMPATIBILITY OF ANTIBIOTICS WITH OTHER AGRICULTURAL CHEMICALS

It is frequently expedient, if not absolutely necessary, to apply several chemicals to plants at the same time. Furthermore, it is equally important that each of the chemicals applied reach the plant and remain there for a time in an active condition. If two or more active ingredients are combined to formulate an effective spray or dust, they are said to be compatible.

Compatibility implies a number of conditions: (a) that the active ingredients do not react with each other to destroy or reduce their respective effectiveness; (b) that the combination does not impair their rate of reaction with the pest; and (c) that the combination is non-injurious to the host plant.

Before antibiotics could be used on a commercial basis in sprays, dusts, dips, etc., their compatibility with other agricultural chemicals had to be established.

In this regard, Ark²¹ reported that laboratory tests revealed streptomycin to be compatible with arasan (tetramethyl thiuramdisulfide), fermate (ferric dimethyl dithiocarbamate), captan (n-trichlormethylmercapto-4-cyclohexene-1,2 dicarboximide), sulfur, lime sulfur, copper, DDT (1:1:1-trichloro-2:2-di (p-chlorophenyl) ethane), ovotran (p-chlorophenyl p-chlorobenzenesulfonate), malathion (S-(1:2 dicarbethoxyethyl)-O, O-dimethyl phosphorodithioate), dimite (di(p-chlorophenyl)

ethanol), aramite (butylphenoxyisopropyl chloroethyl sulfite), and parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate). Dye¹¹² expanded this list of agricultural pesticides compatible with streptomycin to include lindane (hexachlorocyclohexane), DDD (tetrachlorodiphenyl ethane) and a 1:2:100 Bordeaux mixture (1 pound copper sulfate, 2 pounds hydrated lime and 100 gallons of water). No incompatibility was noted by Clayton⁶¹ between either streptomycin sulfate or nitrate and ferbam, captan, phenyl mercuric acetate or parathion in field tests where streptomycin was being used to control fireblight (*E. amylovora*). Palm and Young²⁴² conducted *in vitro* compatibility trials to determine whether or not dichlone (2:3-dichloro-1:4-naphthoquinone), captan and maneb (manganous ethylene bisdithiocarbamate) were compatible with streptomycin and terramycin. Using *Bacillus subtilis* and *Fusarium roseum* as test organisms, they found dichlone and captan to be compatible with streptomycin. The authors also reported that terramycin lost activity immediately upon combination with maneb and that the fungicidal activity of a streptomycin-maneb mixture decreased significantly after seven days. Studies in the control of bacterial and fungal decay of potato seed pieces²³⁵ revealed that nabam (disodium ethylene bisdithiocarbamate) and the sodium salt of orthophenyl phenol did not influence the effectiveness of streptomycin in controlling *E. atroseptica* and *P. fluorescens*. They also noted that good control of fungus rot (associated with streptomycin treatments) could be attained by whole tuber treatments with zineb (zinc ethylene bisdithiocarbamate), dichlone and captan. This treatment was made prior to cutting and treating the potatoes with streptomycin and was compatible with the antibiotic. Ark reported²⁶ that streptomycin in combination of 1 or 2% lime-sulfur (pH 10.5 and 10.7), and streptomycin with hydrated lime (pH 12.7) did not affect stability of streptomycin for at least 2 months. However, streptomycin combined with undiluted lime-sulfur lost antibiotic activity in 24 hours. Efforts to control powdery mildew of red raspberry, *Sphaerotheca humili*, with 5 mcg/ml actidione by Fulton,¹²³ were believed to be deleteriously affected due to inactivation of the antibiotic by a lime residue in the spray tank. Leben and Keitt,²¹⁶ studying the stability of the antifungal antibiotic, antimycin, reported that D.D.T., benzene hexachloride and T.E.P.P. (tetraethyl pyrophosphate) had little effect upon the activity of this antibiotic.

E. ANTIBIOTIC BIOASSAYS

The antibiotic bioassay has become for the plant scientist a laboratory technique with at least three important applications. First, and perhaps most obvious, is its importance to regulatory agencies as a method

whereby residues of these chemicals can be detected in extremely small quantities. Since the public health hazard that these drugs may potentiate when consumed regularly in small quantities is of considerable importance, the subject is treated at length elsewhere in this book.

The second significant application of the antibiotic bioassay is its excellence as a tool with which the phytopathologist can determine whether or not the treated plant part contains the minimum inhibitory level of antibiotic within its tissues to prevent infections or eradicate an established disease. This usage may be extended to establish the persistence or stability of the toxicant *in situ*.

Third, and of most basic importance, the bioassay may be utilized to study the nature of the uptake of large organic molecules by plants. This is a phase of plant physiology which is intensely important to the phytopathologist, nutritionist, auxinologist and entomologist. The antibiotic may be regarded in this connection as a "biological tracer," and so seems to be second only to radioactive tracers as a means of studying in plants such phenomena as adsorption, penetration, absorption and translocation of comparatively large organic molecules.

1. TYPES OF BIOASSAYS

In general, there are three types of bioassays that are commonly used to measure the concentration of antibiotics in plant tissue. All have one principle in common, which is that the growth or lack of growth of the test organism is a measure of the quantity of antibiotic present in the tissue sample.

a. Direct or Tissue Diffusion Assay

Perhaps the simplest bioassay method currently employed utilizes an agar plate seeded with the test organism, upon which is placed a piece of plant tissue suspected to contain an antibiotic. If an antibiotic is present in the tissue, it will diffuse into the agar, preventing the growth of the test organism. This method is evidently not quantitative, but it does permit the qualitative detection of the presence of antibiotic activity. The author has used leaf discs of apple and coleus obtained with the aid of a cork-borer, and cylinders of carnation stem tissue in antibiotic translocation studies (see Figs. 4-3 and 4-4). Leben and Keitt²¹⁶ have described an excellent, and fairly quantitative assay, using leaf discs, for antimycin. Mirzabekyan^{250,251} placed pulverized tomato seedlings on plates seeded with *Corynebacterium michiganense*, to establish positively the penetration of streptomycin and penicillin into seeds and their subsequent movement into the young seedlings. Detached leaves

of *Sedum purpureum* were used by Lockwood²³² to study streptomycin absorption by foliage, because the leaf epidermis of this plant is easily stripped away. Leaf discs cut from sprayed leaves were frozen, the epidermis was removed, and the discs were placed on agar seeded with *A. tumefaciens*. Sensitivity of the assay was reported to be 0.1 mcg/ml. In much the same fashion, Sutton and Bell³²⁶ placed swede seed on agar plates seeded with *X. campestris*, and were able to detect the presence of chlortetracycline in the seed. Alcorn and Ark⁶ made good use of the technique with stem sections of pyracantha and with carnation cuttings on plates seeded with *E. amylovora*, in their studies of the movement of streptomycin and oxytetracycline in plants. Using *Saccharomyces pastorianus* as the test organism, Wallen and Millar³⁴⁸ were able to detect cycloheximide activity when the leaves of wheat seedlings were laid on the seeded agar.



FIGURE 4-3. LEAF DISC ASSAY. Coleus leaves treated with streptomycin. Discs were placed on an agar-plate seeded with *Bacillus subtilis* (ATCC 6633).

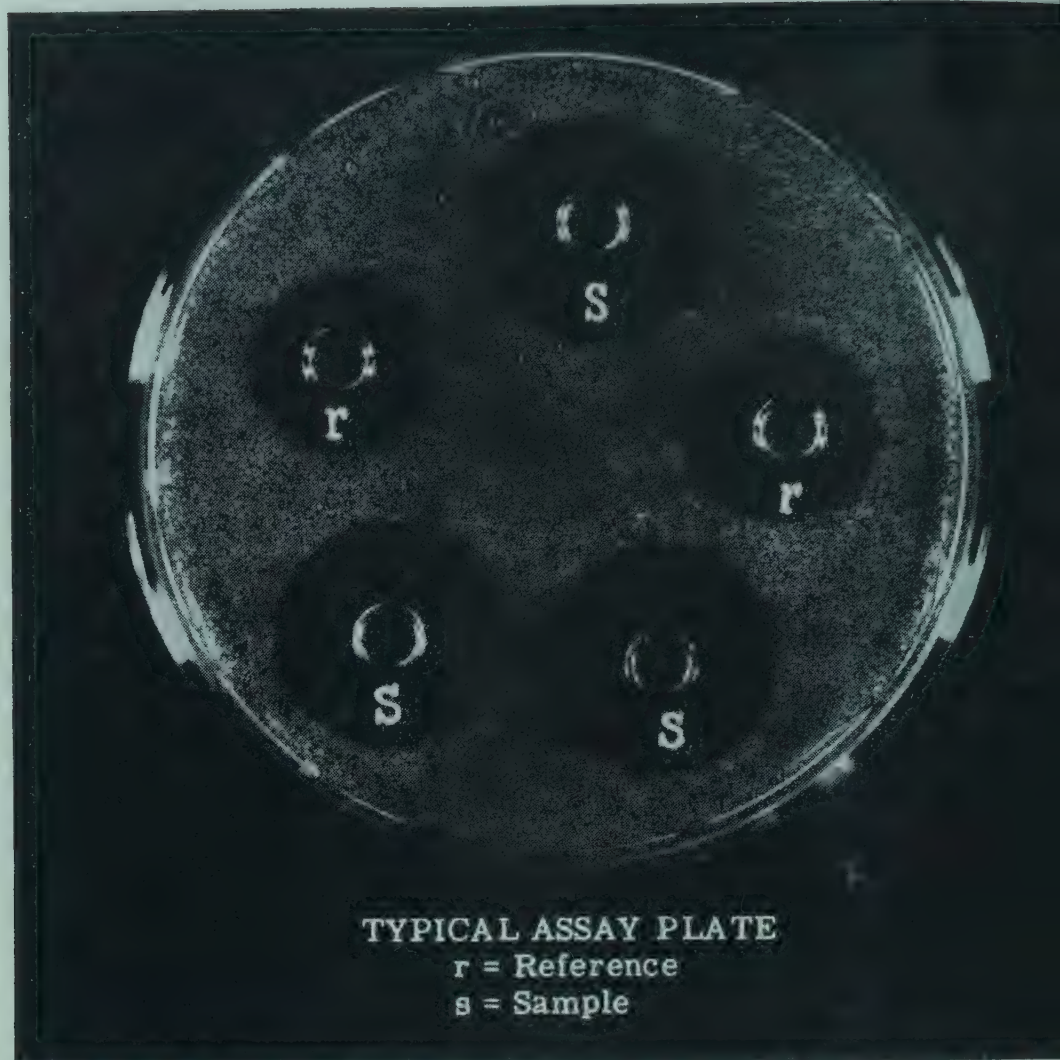


FIGURE 4-4. CYLINDER ASSAY. Cylinders containing oxytetracycline set on agar seeded with *Bacillus cereus* var. *mycoides* (ATCC 9634).

b. Liquid Media Assays

Assays of this type utilize the expressed juices or filtered homogenates of plant tissue as samples. There are two essentially different liquid media assays, the tube-dilution and the turbidimetric procedures.

In the tube method, serial two-fold dilutions are made, usually in 8 to 10 tubes, so that the sample is diluted by one-half 8 to 10 times. These serially-diluted tubes are then inoculated and allowed to incubate for a suitable period. The highest dilution of the sample that inhibits growth of the test organism is determined visually and compared with the inhibiting dilution of known concentrations of the standard. This method has been used by the author^{143,255} to assay apple and potato tissues

The method has two serious disadvantages, and as a result is no longer used in our laboratories. First, it provides only an approximation of the amount of antibiotic present in the sample, because only a range of activity is detected. The second disadvantage is that the material to be assayed must be clear; or otherwise the end-point of the assay may be obscured by a turbidity inherent in the sample.

In the turbidimetric method, the antibiotic-containing plant extract is diluted in a suitable substrate, such as peptone, inoculated with the test organism, incubated, and the turbidity is measured with a colorimeter or spectrophotometer. The per cent light transmission or the optical density is then plotted on a standard curve prepared from a series of inoculated tubes containing a range of concentrations of the pure antibiotic. This procedure is evidently more accurate than the tube method, but it has the same disadvantage in that turbid samples interfere with the assay. The preparation of clear samples for assay, even after filtration through U.F. sintered glass filters, is difficult for some plant tissues, due to precipitation of colloidal matter during the incubation period.

c. Agar Plate Method

Most workers have adopted variations of the agar plate diffusion method. The antibiotic-diffusing surface in this test may be a standardized filter paper disc, a porcelain or stainless steel cylinder, or merely a well in the agar, formed by removing an agar disc with a cork-borer. Each of these surfaces can receive a measured amount of sample, which can then diffuse into the seeded agar. The quantity of antibiotic in the sample is calculated by measuring the diameter of the zone of inhibited growth in the seeded plate. This figure is plotted against a standard curve prepared from a series of concentrations of the pure antibiotic. (At times, however, it has been found desirable to prepare the standard curve by using extracts from untreated plants of the type being studied. This procedure compensates for any interference inherent in the sample.) Lockwood *et al*²³⁰ have developed a variation of this method of assay in which they have substituted a large metal tray for the conventional petri dish. The seeded agar in the large tray responds similarly to seeded agar in plates, and, in addition, permits many more samples to be evaluated at one time. In fact, a standard curve can be prepared on each tray. An advantage of the agar assay over the liquid media assays is that a number of plant components which interfere with the liquid assays do not diffuse in agar, and hence do not affect the assay deleteriously.

For a more detailed description of assay methods, the reader is directed to a recent text on the subject by Grove and Randall¹⁶⁴ which currently serves as the manual of official methods for the Antibiotics Division of the Food and Drug Administration.

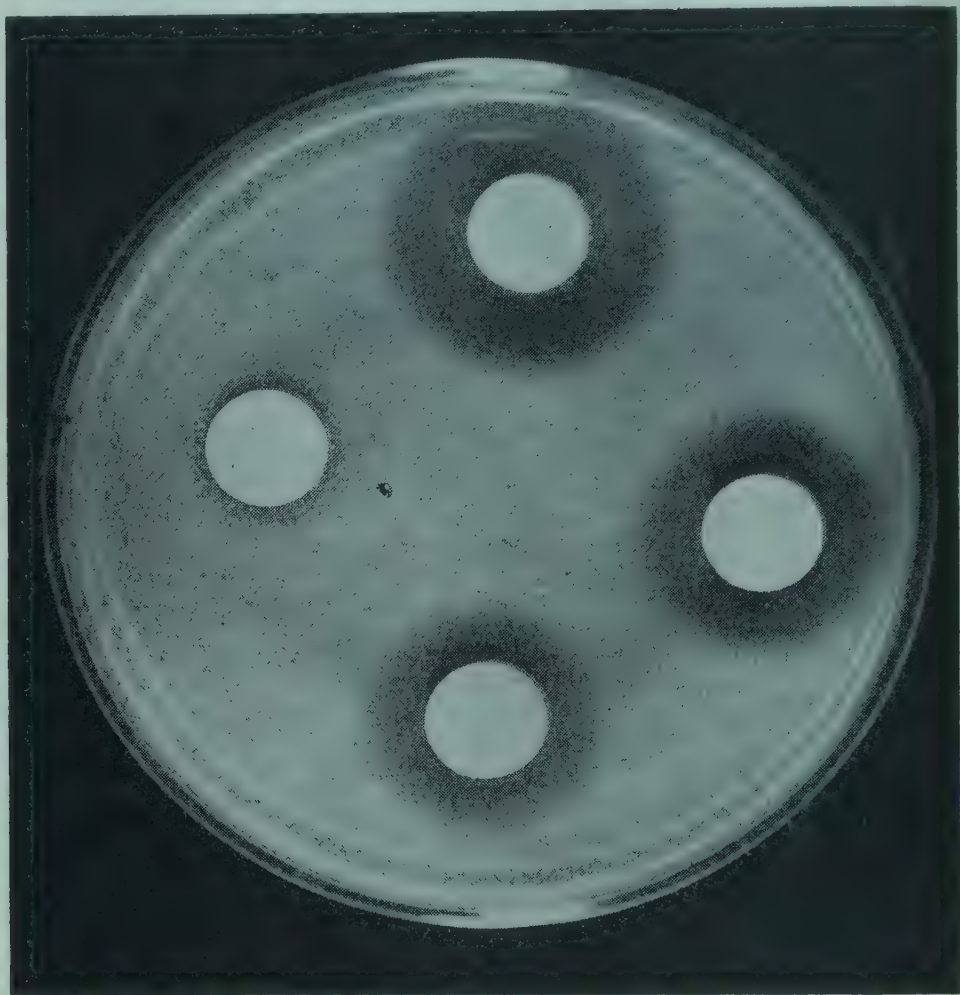


FIGURE 4-5. FILTER PAPER DISC ASSAY. Filter paper discs containing 1, 5, 10, and 20 mcg/ml oxytetracycline on agar seeded with *Erwinia amylovora*.

The filter paper disc-agar diffusion method has been most widely used by phytopathologists (see Fig. 4-5). It is rapid, easily performed, and if samples are sufficiently replicated, the results are quite reproducible. Crossan and Krupka⁷⁷ employed this method in attempts to detect a streptomycin-oxytetracycline formulation in pepper foliage, using *X. vesicatoria* as their test organism. This organism is not particularly sensitive and is a comparatively slow grower. The sensitivity of this assay can be vastly increased by using *Bacillus subtilis* (ATCC 6633) as the

test organism for streptomycin, and *B. cereus* var. *mycoides* (ATCC 9634) for the tetracycline antibiotics. In our laboratory, the bioassay sensitivity for streptomycin is 0.1 mcg/ml, and for oxytetracycline and chlortetracycline it is 0.05 and 0.005 mcg/ml, respectively. Stainless steel cylinders are employed as a routine procedure, and the official methods of Grove and Randall¹⁶⁴ are closely followed, with minor changes in inoculum and sample preparation (see Fig. 4-4). Cox *et al.*,⁷⁴ using the above method for oxytetracycline, reported a sensitivity of 0.03 mcg/ml.

Gray¹⁵⁸ has had considerable success with the filter paper disc bioassay for streptomycin, using a streptomycin dependent strain of *E. coli* MB 464. In this assay the relative quantity of streptomycin is measured by the amount of growth in the seeded plate; the greater the quantity of streptomycin, the more the amount of growth that is apparent.

TABLE 4-3

THE EFFECT OF POTASSIUM SALTS ON THE CONCENTRATION
OF STREPTOMYCIN IN THE SUPERNATANT LIQUID OF A
CARNATION STEM TISSUE HOMOGENATE

Treatment	Conc. mcg/ml
4 mcg/ml strep. in pH 8 buffer*	2.00
4 mcg/ml strep. in 1% KCl	1.27
4 mcg/ml strep. in distilled H ₂ O	.28

* Phosphate buffer contained 16.73 gms K₂HPO₄ and 5.23 gms KH₂PO₄/liter.

Gottlieb and his group^{150,151,240,241} have used the paper disc method effectively in their streptomycin adsorption studies.

In conducting some bioassays on peach leaf tissue for streptomycin, Dye^{111,113} observed that by adding killed peach leaf tissue to streptomycin-containing solutions, the amount of streptomycin detected in the supernatant liquid was decreased. To explain this phenomenon, Dye suggests that the streptomycin may be bound to the peach tissue. The author submits that this phenomenon may be quite analogous to the partially reversible binding of the positive streptomycin molecule (S⁺) to the negatively charged pyrophyllite clay (C⁻). To support this contention the author presents, in Table 4-3, previously unpublished data.

The above experiment indicates that the K⁺ ions compete with the S⁺ molecules for sites on the negatively charged plant tissue. The greater effectiveness of the phosphate K⁺ ions than the chloride K⁺ ions may be more apparent than real, since there were more dissociating K⁺ ions in the phosphate buffer solution.

d. Hyphae Curvature Assay

The antibiotic assay for griseofulvin differs considerably from those previously described. This assay was first described by Brian *et al.*,⁴⁴ and involves the preparation of serial dilutions with equal quantities of a suspension of *Botrytis allii* conidia in nutrient solution and the observation of their germination after an 18-hour incubation. By comparing the characteristic distortions of growth of the hyphae with those produced in solutions of known strength, concentrations of griseofulvin in plant tissue can be accurately estimated.

2. SAMPLE PREPARATION

Brian and his group^{44,46,82,323} have frequently bioassayed guttation water from plant foliage in their studies regarding the systemicity of griseofulvin. It is the opinion of the author that this type of sample should be ideal for a number of reasons. Obviously it might reflect directly the concentration of free antibiotic in the plant tissues, barring, of course, concentration upon evaporation. The sample is easily collected; it is free of tissue and other solids; and it should, therefore, contain less interfering substance than a homogenate or expressate.

Generally, workers in this field have obtained cellular liquids for assay either by homogenizing or by expressing the juices from tissue killed by freezing.

Homogenates have proved successful where the antibiotic being sought is not water-soluble and the appropriate organic solvent is used in homogenization, as shown by Crowdy and Pramer.⁸⁰ Phosphate buffers have also been used successfully in this way by Dye.¹¹¹ Aqueous homogenates may be assayed directly. However, if liquid media assays are to be performed, passage of the sample through sintered glass (ultra-fine) filters or centrifugation of the specimen is usually obligatory.

In our laboratory the tissue expressate has been found most satisfactory, since the antibiotics of primary concern are the water-soluble tetracyclines and streptomycin. If the tissue is frozen prior to pressing, considerable liquid is released, with a minimum of solid material. The standard curve solutions are prepared in expressate from non-treated plants, to which known concentrations of the antibiotic are added, and care is taken to remove all external moisture from plant tissue prior to pressing.

3. VALIDITY OF ASSAYS

It is necessary to establish rather conclusively that the antimicrobial activity detected in plant tissue by a bioassay is in fact the

antibiotic which has been applied. There are other possible sources of activity which may obscure the issue. For example, the antibiotic while inside the plant may be transformed by a metabolic process. Another possibility is that the plant being assayed may contain an indigenous compound which is active antimicrobially. Certain quinones, aglycones and alkaloids are known to display activity of this nature.

In order to establish, with as much certainty as possible, the identity of the antibiotic detected, some workers have employed corroboratory techniques. Mitchell *et al*²⁵⁴ inoculated, with a streptomycin-dependent strain of *E. coli*, the zones of inhibition caused by the antibiotic they had recovered from their streptomycin-treated bean plants. The fact that the organism grew in this area provided additional proof that the activity detected was due to streptomycin.

Crowdy *et al*⁸² were of the opinion that bioassays alone did not positively identify the antibiotic, believing that actual recovery of the applied compound and identification of it through chemical analyses were necessary to validate the assay. They showed that countercurrent distribution in a suitable solvent system, coupled with chromatography on an activated alumina column, could effect quantitative separation of both griseofulvin and chloramphenicol from bean plant tissue. The above method isolated amounts of both antibiotics which compared favorably with estimates made spectrophotometrically. Furthermore, results from these methods were also in agreement quantitatively with estimates made through bioassays. Thus, the chemical tests not only established the identity of the active principle, but also validated the accuracy of the bioassay in question.

When post-fermentation modification of cycloheximide to produce the oxime, semicarbazone, or acetate form of this antibiotic was accomplished, the question regarding the active form of the antibiotic was immediately raised. Hamilton and his associates¹⁶⁸ had reported that these newer derivatives are more effective and less phytotoxic than the original compound. However, they stated that it was not yet known whether the cycloheximide derivatives remain active in their modified forms, whether they are altered by some metabolic process of the plant, or whether they revert to the original molecular configuration of cycloheximide after they are inside the plant. An answer to the question posed by Hamilton was forthcoming from the experiments conducted by Wallen and Millar.³⁴⁸ They were able to determine through paper chromatography that the active substance from chloroform extracts of wheat plants grown in solutions of cycloheximide and the pure antibiotic had Rf values of 0.61 and 0.59, respectively. Similar procedures compared guttation fluid from treated plants with aqueous solutions of the

pure antibiotic and showed identical Rf values of 0.59. From these experiments the authors concluded that the active principle in the plant samples is either cycloheximide or a closely related compound. The question appears now to have been answered conclusively by the work of Lemin and Magee.²²⁵ They utilized cycloheximide acetate labeled by a radioactive carbon atom (C¹⁴) in the number two position of its acetate radical; and they used paper chromatography in their analyses. Their data suggest that cycloheximide acetate appeared as such only in the roots of treated plants, and that activity detected in the leaves of these plants was due to cycloheximide *per se* (see Table 4-4).

TABLE 4-4
ANTIFUNGAL ACTIVITY IN THE TOMATO TISSUE DERIVED
FROM CYCLOHEXIMIDE ACETATE-2-C¹⁴*

	Equivalent total mcg activity in the extracts (two plants)		
	4 hours	7½ hours	11 hours
Alcohol extract of root	102	107	168
Alcohol extract of stem	115	404	413
Alcohol extract of leaves	133	404	1023

* No *in vitro* activity of cycloheximide acetate using levels up to 1000 mcg/ml has been observed in this bioassay. The equivalent total mcg was calculated using cycloheximide as a standard. (After Lemin and Magee.²²⁵)

Colorimetric, infrared and ultraviolet spectrophotometric and other chemical methods of assay for a number of the more prominent antibiotics are described in detail by Grove and Randall.¹⁶⁴

F. ADDITIVES TO ANTIBIOTIC FORMULATIONS

Up to the present time, antibiotics employed to control plant disease under field conditions have been used most commonly in sprays, and to a lesser extent, in the form of dusts. Of these two methods the foliar spray has been by far the most popular and successful.

It is generally conceded that the primary reason for the importance of antibiotics in the plant grower's arsenal of pesticides is the systemicity of a number of these compounds. However, both foliar sprays and dusts have a decided limitation in that the levels of antibiotic attained in plant tissue by these routes of administration are characteristically low.

Investigators have evaluated a number of chemical additives used to improve the quality of systemicity and to increase the antibiotic level

within plant tissues. These additives are exceedingly diverse, since they are directed at specific morphological, physiological, and biochemical characteristics of plants, characteristics that are believed to influence penetration, absorption, and translocation of organic chemical compounds by foliage.

Although our understanding of cationic and anionic absorption through plant roots is rapidly and significantly expanding,^{50a,117} very little is known of foliar absorption. It has not been too long since even relatively simple organic compounds were first introduced into plants through their foliage. Foliar feeding of urea as a nitrogen source is a comparatively recent development. Contrast the molecular weight and configuration of urea and streptomycin, the latter having a molecular weight of nearly 600, against a value of 60 for the former.

Large organic molecules face a number of possible barriers to their entry into plant cells. Where the cuticle is present, this wax-like layer on the leaf surface may impede passage. Next comes the cell wall, followed in order by the outer plasma membrane, the protoplasm itself, the inner plasma membrane or tonoplast, and entry into the vacuole of the cell.

The attainment of antibiotic levels inhibitory to plant pathogens through foliar applications is made still more difficult, theoretically at least, by translocation of the drug away from the original place of absorption. Further, it is conceivable that cellular levels of antibiotics applied to rapidly-growing plant tissue may be decreased through dilution.

To obviate, or at least to circumvent these barriers, theoretical and real, surfactants, humectants, growth regulators, inorganic salts, organic solvents and heavy metals have been added to antibiotic formulations.

1. SURFACTANTS

In order to reduce surface tension, and thereby spread the antibiotic uniformly over the leaf surface, Triton B-1956 (phthalic glycerol alkyd resin) and Tween 20 (polyoxyethylene sorbitan monolaurate) along with other surface-active agents have been added to antibiotic sprays.^{7,8,221} Young and Fulton³⁶⁵ reported that Triton B-1956 appeared to increase the efficacy of cycloheximide against powdery mildew of dewberry. It is well known, however, that the mycelia of the powdery mildews are extremely difficult to wet, and for this reason the surfactant gave better results by producing improved contact of the antibiotic with the fungus. On the other hand, Gray¹⁵⁸ reported that surfactants were generally without effect on the absorption of streptomycin. He did, however, indicate that the beneficial effect of streptomycin-glycerol combination

was reduced by Tween-20. Koontz and Biddulph²⁰⁸ have reported that surfactants in general impaired the absorption of radioactive phosphate applied to foliage. They attributed the reduction in absorption to an increase in the rate of drying of the spray on the leaf surface.

It is of some interest to note that Lockwood and Williams,²³¹ while evaluating streptomycin and oxytetracycline as control agents for *Bacterium stewartii*, showed that Tween-20 at 10,000 mcg/ml significantly lessened the severity of wilt symptoms. Later, these workers³⁵⁵ reported partial inhibition of *B. stewartii* by a number of surfactants.

Using leaf discs of *Sedum purpureum*, Lockwood²³² studied the absorption of streptomycin when applied to foliage as a spray. He observed that 0.1% Tween-20 provided a mean of 49% absorption of the amount applied, whereas a mean of 14% was obtained when water alone was used as the solvent. In opposition to the above results,^{158,208} this increase in absorption was attributed to the surfactant qualities of Tween-20. However, it should be pointed out that this compound is also a hydrophile. Its humectant qualities are due to the free hydroxyl and oxyethylene groups it carries, and these may in fact account for the results obtained by Lockwood.

2. HUMECTANTS

The manner in which relatively large organic molecules are absorbed by foliage is not well understood. Some factors influencing the process are assumed, while others remain unknown. For example, it is not known whether foliar absorption is a matter of simple diffusion or whether it is a process which is actively or metabolically mediated. It is also not known whether these chemicals penetrate primarily through natural openings in the vapor phase or move directly through the cuticle, cell wall, and plasma membrane.

What is apparent, however, is that the vehicle for absorption of these organic molecules is water. Conversely, those factors which favor rapid drying of the spray solution adversely affect absorption. Thus, the longer the antibiotic-containing spray remains wet on the leaf surface, the more time the antibiotic has to enter the plant.

This fact was first considered in our greenhouse experiments wherein streptomycin sprays were formulated as lanolin emulsions, and with Carbowax 4000.^{100,101} Later, Gray^{154,156} demonstrated that glycerol at 1% increased the absorption by bean plants. In additional experiments¹⁵⁸ this increased absorption was noted in tomato, pepper, and tobacco plants. Gray also demonstrated that glycerol increased the absorption of streptothricin and chloromycetin, but had no apparent effect upon the uptake of neomycin, penicillin, or oxytetracycline by plants. We

have recently demonstrated¹⁴⁵ that di- and triethylene glycol are as effective as glycerol in increasing the absorption of streptomycin by bean plants.

In vivo disease control experiments are not all in good agreement regarding the efficacy of glycerol as an adjuvant for streptomycin absorption.

Improved disease control with a glycerol-streptomycin combination has been reported by Krupka and Crossan²¹¹ in experiments designed to control *Xanthomonas vesicatoria*. The beneficial effects of glycerol were also noted in studies conducted by Anderson¹¹ on bean blight, caused by *X. phaseoli* and *X. phaseoli* var. *fuscans*, and by efforts of Daines and Gray⁸⁷ with peach shot-hole, caused by *X. pruni*.

Conversely, Miller²⁴⁵ noted no significant improvement of fireblight, caused by *E. amylovora*, nor did Zaumeyer and co-workers^{368,372} in their

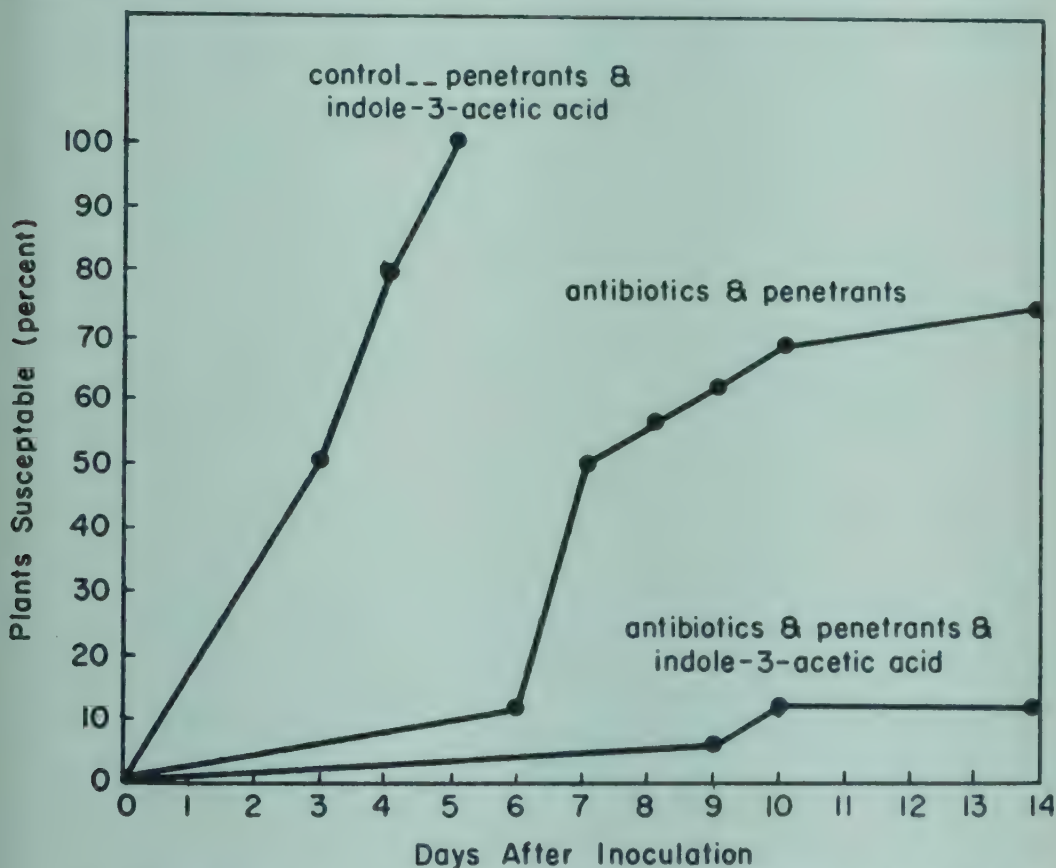


FIGURE 4-6. INFLUENCE OF IAA ON IMPROVING CONTROL OF *E. amylovora* IN ARTIFICIALLY INOCULATED YEAR-OLD APPLE TREES WITH AN ANTIBIOTIC SPRAY. In the above treatments the spray components were as follows: penetrants, 1 percent each carbowax 4000 and Methyl Cellosolve; antibiotics, 250 meg/ml each of streptomycin and oxytetracycline; indole-3-acetic acid at 100 meg/ml.

efforts to control tomato late blight, caused by *Phytophthora infestans*, and downy mildew of lima beans, caused by *Phytophthora phaseoli*.

In absorption experiments, Ark²⁸ reported that dipotassium phosphate reversed the beneficial effect of glycerol. It will be recalled that Gray¹⁴ found a diminution of the effect of glycerol when combined with Tween-20.

3. GROWTH REGULATORS

Quite by accident, in a series of greenhouse experiments, we observed that indole-3-acetic acid (IAA) vastly improved the efficacy of streptomycin in protecting apple shoots against artificial inoculation of *E. amylovora*¹³⁷ (see Fig. 4-6). Subsequently we reported¹⁷⁴ that this effect was not peculiar to IAA, but could be attained with a number of plant

TABLE 4-5
EFFECT OF HYDROXY-COMPOUNDS AND GROWTH REGULATORS
ON THE ABSORPTION OF STREPTOMYCIN (200 MCG/ML)
BY BEAN FOLIAGE

Additive*	Replicates per treatment	mcg/ml Antibiotic detected (average of replicates)
None	8	4.73
Glycerol 1%	7	11.21
TEG 1%	4	11.76
DEG 1%	2	13.02
NA 20 mcg/ml	6	11.20
GA 30 mcg/ml	2	11.58
IAA 100 mcg/ml	2	3.10
IAA + Glycerol	2	4.08

* TEG = triethylene glycol; DEG = diethylene glycol; NA = naphthyl acetamide; GA = gibberellic acid; IAA = indole-3-acetic acid. (After Goodman and Dowler.¹⁴⁵)

growth-regulating substances. It was noted that the ethyl ester of IAA and naphthyl acetamide (NA) were particularly effective in this respect. Our most recent investigations¹⁴⁵ indicate that both naphthyl acetamide and gibberellic acid (GA) increase the absorption of streptomycin by bean plants. This increase is of the same order obtained with glycerol, and with di- and triethylene glycol. Paradoxically, these experiments revealed that IAA, which improves the performance of streptomycin *in vivo*,¹⁴⁵ actually depresses streptomycin absorption and, in addition, completely reverses the beneficial effect of glycerol (see Table 4-5).

An antibiotic-auxin interaction was previously reported by Iyengar and Starkey,¹⁹³ who showed that oxytetracycline, chloromycetin and, to some extent, streptomycin synergize the effect of IAA (auxin) on *Avena* coleoptiles.

4. ADSORBANTS

The earliest field experiments successfully employing antibiotics formulated as sprays and dusts to control a fruit tree disease were conducted by Ark in 1949 and 1950.¹⁹ These efforts showed streptomycin to be exceedingly effective, at the extremely low concentration of 1 mcg/ml, in controlling walnut blight, *X. juglandis*. In his 1950 experiment, Ark explored the possibility of adsorbing the positively-charged streptomycin molecule (S^+) on a synthetic resin IRC-50 and applied this adsorbed form of streptomycin as a dust to walnut trees. The intent here undoubtedly was to provide a slow liberation of streptomycin by the resin in the presence of atmospheric moisture. The results obtained were most gratifying and promising. Garber *et al*¹²⁶ subsequently prepared streptomycin in a number of dust forms of low water solubility. These were applied to tomato foliage, which in turn was subjected to simulated "dew," and under these conditions appreciable amounts of streptomycin were released. Ark and his group,^{18,23,24,26,28,29} also formulated streptomycin with a number of clays possessing a 2:1 lattice structure. In some of these, however, streptomycin was adsorbed irreversibly, and in others release of the antibiotic could only be effected through an exchange reaction. In the latter instances K_2HPO_4 supplied the K^+ ion to remove the streptomycin (S^+) from the negatively charged colloidal clay particles. Further work by Ark disclosed the fact that the bentonite (Montmorillonite or expanding 2:1 lattice) clays were not suitable diluents for streptomycin, whereas the pyrophyllite clays were more effective for this purpose.

5. CATIONIC EFFECTS

Just as streptomycin was adsorbed by the negatively-charged clay particles to form dusts, one might expect similar reactions of some antibiotics at the negatively-charged colloidal surfaces of plant cells.

The cell wall, protoplasm and plasma membranes are essentially aqueous colloidal dispersions. It is apparent, therefore, that the colloidal micelles comprising these membranes could adsorb positively-charged streptomycin, streptothricin, neomycin molecules, the polypeptide antibiotics and the amphoteric tetracyclines.

As a result of the exchange reactions that some antibiotics are able to undergo, it is not surprising that these drugs have also been found to interact with mono- and divalent cations at plant surfaces. Alcorn and Ark^{5,6,26} reported that dipotassium phosphate increased the acropetal movement of streptomycin, and they suggest that a base exchange is involved. However, neither Mn^{++} nor IAA were effective in this respect.

These authors⁶ also present evidence that $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ at 5 times the molar concentration of streptomycin did not reverse the inhibitory effect of the antibiotic on root formation of carnation cuttings. The manganous ion has been described as reversing the inhibitory effect of streptomycin on roots of tomato plants by Gray,¹⁵³ who noted approximately 5 times as many moles of Mn^{++} as streptomycin were needed for maximum activity. Rosen³⁰⁹ has shown that 10^{-3} M Mn^{++} could prevent the inhibitory effect of 100 mcg/ml of streptomycin on the growth of *Avena* coleoptiles. Chloride salts of Na^+ , Mg^{++} , Ni^{++} , and Co^{++} were without effect; however Ca^{++} was approximately 50% as effective as Mn^{++} . In exchange experiments using barley roots, Norman²⁷³ relates that 5 mcg/ml of the polypeptide antibiotic, polymyxin, depressed the growth of roots where the Ca^{++} concentration was 10^{-5} , 10^{-4} , and partially at 10^{-3} . However, at a concentration of Ca^{++} of 10^{-2} this inhibition was annulled.

6. SOLVENTS

The fact that the cuticle on the surfaces of some leaves interferes with factors affecting the success of foliar sprays is readily apparent. It is known that cutinized cell walls are relatively impermeable to water, and the reason for this might be more apparent if the composition of this layer was described. The cuticular layer is viewed by Frey-Wyssling¹²² as being a mixture of the four cell-wall constituents. These are cellulose and pectins which are hydrophylic compounds, cutin which is semi-hydrophobic, and the waxes which are of course lipophilic. Of particular interest here is the proposed arrangement of these constituents. On the inner face of the cuticular layer, cellulose and pectins predominate, while the other layers are predominantly cutin and cutin-waxes.

The foregoing discussion portrays the cuticle as a formidable barrier to aqueous solutions of organic compounds applied to foliage. It was for this reason that the early antibiotic sprays were formulated with methyl cellosolve, an organic solvent, which might partially dissolve or perforate the waxy layer of the leaf surface.^{135,136}

Altman and Bachelder⁸ found that streptomycin injury of a number of plants was heightened by the addition of 1% methyl cellosolve to their spray formulations. They, too, reasoned that this phytotoxicity was due to a reduction of cuticle as a barrier. Methyl cellosolve has been used similarly by Ogawa and Vergara²⁷⁴ in experiments evaluating actidione as an eradicant and protectant against *U. necator*, which causes powdery mildew of grapes, and *S. fructicola*, which causes peach brown rot. Leben *et al*²²¹ also used this compound as a carrier of helixin B in treating oat seeds for Victoria blight, caused by *H. victoria*. It would

seem that Miller's²⁴⁵ application of kerosene at 500 ppm, together with streptomycin in efforts to control *E. amylovora*, was based on the lipid solubility of the additive.

7. EFFECT OF HEAVY METALS

Perhaps the most widely used of the heavy metals for disease control purposes is copper. The mechanisms regarding its fungitoxicity and bactericidal qualities have been fairly well worked out. For a comprehensive review of the literature regarding copper as a microbial toxicant, the reader is directed to a recent book by Horsfall.¹⁸⁹

Copper sprays in the form of Bordeaux mixture, and as copper sulfate, have been the standard protective agents against many bacterial diseases, including those due to *X. juglandis*, *E. amylovora*, *X. vesicatoria*, *P. tabaci*, and *P. apii*.

In efforts to control bacterial blight of celery in 1955, Cox⁶⁹ noted that a combination of Agrimycin (a 15%-1.5% streptomycin-oxytetracycline combination) and metallic copper (45%) provided better results than either of the antibiotics or the copper alone. These results were soon repeated by Cox⁷⁰ in experiments to control bacterial spot of pepper, caused by *X. vesicatoria*, and since then has refined this treatment for commercial use.^{71,72,75} The results of Cox were soon extended by Coe,⁶³ who also found an additive effect of streptomycin and copper, in efforts to control the downy mildew fungus of cucurbits, *Pseudoperonospora cubensis*. Similarly Zaumeyer and West³⁶⁸ have reported that control of downy mildew of lima beans, caused by *Phytophthora phaseoli*, is improved by the streptomycin-neutral copper combination. They found that sprays containing 25 mcg/ml streptomycin and 25 mcg/ml neutral copper were more effective than those containing 50 mcg/ml of neutral copper and slightly better than 50 mcg/ml of streptomycin. The antibiotic at 25 mcg/ml afforded no protection against the fungus.

As with most research findings, unanimity of opinion is a rarity, and the reports of Shaw *et al*^{314,315} are examples of this fact. The authors reported that tribasic copper sulfate decreased control of *P. tabaci* when added to streptomycin.

Of interest here is the report of Crosse⁷⁹ that in-bloom sprays of streptomycin are particularly effective in controlling bacterial canker of cherry, caused by *Pseudomonas mors-prunorum*. Streptomycin is suspected by Crosse as being effective during this period against the leaf spot-phase of the disease, which is a source of inoculum for the canker stage. In his experiment Crosse has found Bordeaux mixture more effective than streptomycin, when applied late in the season as a canker

preventative. His conclusions are that Bordeaux mixture is a better surface disinfectant than streptomycin. However, streptomycin applied early is viewed as a systemic protectant reducing the inoculum potential.

G. PHYTOTOXICITY

Since fungi and bacteria are considered lower forms of plant life, it is not surprising that substances which are toxic to them should act similarly upon the higher plant forms. One might even predict that the margin of safety for the use of most of these toxicants is exceedingly small. This prediction appears to be well founded for a number of antibiotics, and, in addition, the margin narrows appreciably for some species of plants. For example, Swartwout³²⁷ has reported that cycloheximide is phytotoxic to some hybrid tea rose varieties at 0.1–0.2 mcg/ml, and Pound and Stahmann²⁸⁰ have reported tomato cuttings to be sensitive to .05 mcg/ml of alternaric acid.

The term phytotoxicity is an all-encompassing one which describes effects detrimental to the normal growth and development of plants. These effects may be large or small, visible or invisible; for example, the injury described by Swartwout was a foliar modification, a chlorosis, that is, a suppression of chlorophyll. It is known that higher concentrations of cycloheximide produced symptoms that were more severe, the chlorosis becoming more intense and affected larger areas. What is unknown, however, and can only be surmised, is that concentrations lower than 0.1–0.2 mcg/ml may have produced more subtle effects.

The literature, as is shown below, contains numerous accounts of antibiotic-induced phytotoxicity.

Most reported toxicities were readily apparent to the unaided eye, and thus reflect profound cellular modifications. It is, therefore, interesting to speculate upon the possibility that smaller amounts of antibiotic could have produced effects proportionately less severe. One might find that antibiotics at effective levels (antipathogenic) are generally toxic to both host and pathogen, but more so to the former than to the latter.

Factors which may be considered as affecting significantly the type and severity of phytotoxicity manifested are the plant species and plant part treated, the antibiotic used, the dosage and length of exposure, and the environmental factors which influence absorption. Specific details of phytotoxic effects and their implications are discussed separately for each antibiotic.

1. STREPTOMYCIN

Since streptomycin is the antibiotic most widely used in plant pathology, more is known about the phytotoxic symptoms it produces.

According to Rosen,³⁰⁸ these symptoms include root thickening, lack of root branching, stem shortening, reduced leaf expansion and chlorosis.

Chlorosis, yellowing, or chloroplast bleaching, as the symptom has variously been called, is perhaps the most commonly observed injurious effect. The condition was first observed by Anderson and Nienow¹² on radish foliage that had been grown from seed soaked in streptomycin at 200 mcg/ml for 12 hours. Shortly thereafter, Von Euler³⁴⁰ treated



FIGURE 4-7. STREPTOMYCIN-INDUCED CHLOROSIS ON APPLE LEAVES.

germinating seeds with 0.2% streptomycin, and noted a bleaching effect on the foliage as a result of the treatment. Using carrot cylinders to study the effect of streptomycin on crown gall tumors, De Ropp⁹⁶ noted that the antibiotic not only reduced the rate of tumor growth, but in addition the treated disc became white, whereas the controls remained green. These brief descriptions of the affect of streptomycin on chlorophyll development were probably precursors to an interesting study conducted by Bogorad.³⁷ Pine seeds were germinated in darkness on an

agar medium containing 0.2% streptomycin, and it was found that the cotyledons of the seedlings developed without chlorophyll. The cotyledons of these seedlings when detached and maintained in the light on a streptomycin-free substrate proceeded to develop chlorophyll.* From these studies one might deduce that streptomycin does in fact suppress chlorophyll development, but that this effect does not persist once the streptomycin source is denied. It is shown below that the former premise is true, but the latter may not be.

The most logical plant to select to study streptomycin-induced chlorosis on the cellular level is a large single-celled algae. This was done by Pringsheim and Pringsheim,²⁹⁵ who treated *Euglena gracilis* with 200–2,500 mcg/ml of streptomycin, which caused some cells to

TABLE 4-6
EFFECT OF STREPTOMYCIN ON NUMBER AND TYPE OF
COLONIES OF EUGLENA IN AGAR MEDIUM

Streptomycin mg. per ml.	No. colonies per plate	Per cent colonies		
		Green	White	Yellow
0.0	370	100	0	0
0.5	396	91	5	4
1.0	440	15	77	8
2.0	476	0	100	0
3.0	417	0	100	0

(After Robbins *et al*³⁰⁶).

become irreversibly apoplastidic (colorless). Of some interest here is the fact that the strains of the algae that could be bleached by heat reacted similarly to streptomycin. Thus, the differences in sensitivity of cell constituents (chloroplasts) are inherent characteristics of the individual strains of algae. Robbins *et al*³⁰⁶ confirmed this mutagenic effect by demonstrating that the action of streptomycin on *Euglena* is not a selective one wherein the rare chlorotic individuals in a green stock culture are favored. Quite the contrary, they showed that by increasing the concentration of streptomycin from 0.5–2.0 mcg/ml, more colonies in a given population are irreversibly bleached. They demonstrated also that this effect was not an all-or-nothing one, but rather a concentration variable phenomenon, since at intermediate levels of the antibiotic, 0.5–1.0 mcg/ml, some pale green and yellow strains were noted (see Table 4-6).

* Control seedlings germinated in the dark but on a streptomycin-free agar also developed chlorophyll in their cotyledons.

In studies designed to disclose the mode of action of streptomycin, Rosen^{307,308} investigated this phenomenon of chloroplast bleaching in higher plants. He observed, as did Bogorad, that the toxic effect is not on the photosynthetic mechanism alone. This symptom could occur both in the light and in the dark. Rosen concluded from his experiments that exposures of higher plants to streptomycin could result in two general phytotoxic effects: (a) leaf bleaching and (b) growth inhibition. Leaf bleaching has been apparent in a number of greenhouse and field experiments from which the following observations were recorded:

1. Intensity of bleaching increases with concentration.^{8,25,112,135,138}
2. Younger tissue, meristematic tissue, tends to become more chlorotic than older tissues.^{8,143}
3. The chlorotic effect is not generally reversible;^{8,25,143,307} however, there is an account that chlorosis disappeared following an application of nitrogen.¹⁷²

These observations appear to be supported by the work with *Euglena*, which indicates that the intensity of the chlorosis is a concentration effect. They seem also to be confirmed by the experiments of Hunter and Provasoli,¹⁹² who found that in higher plants streptomycin does not affect chloroplasts after they are formed, that is, it interferes with their synthesis rather than their function.

The second major phytotoxic effect attributed to streptomycin is growth inhibition, which may be reflected in the performance of roots, stems, leaves and seeds of plants. It will be recalled that Rosen²⁷² described a lack of root branching, root thickening, stem shortening and reduced leaf expansion. Robinson *et al*³⁰⁷ have reported that streptomycin interferes with the normal rooting of chrysanthemum cuttings, whereas Alcorn and Ark⁶ and Gasiorkiewicz¹²⁷ describe a similar effect upon carnation cuttings. The latter noted that streptomycin-treated cuttings failed to form callus tissue, that the toxic effect of this antibiotic upon rooting could be detected at 1 mcg/ml, and that 20 mcg/ml virtually arrested root formation.

Stunting has also been reported by Altman and Bachelder⁸ and Marlatt.²³⁸ Both investigations were designed specifically to evaluate the sensitivity of a number of plant species to streptomycin. De Ropp⁹⁶ observed that streptomycin inhibited the growth of sunflower stem fragments and concluded that streptomycin is a general inhibitor of embryonic tissue.

Some light is shed on the nature of this inhibition of embryonic tissue by the investigations of Tanaka and Sato,⁴²⁹ who studied the effects of

streptomycin on root tips of *Tradescantia paludosa*. They reported its effect upon mitotic cells to be mutagenic, thus confirming the experiments with *Euglena*. They were able to view clotting, contraction and fragmentation of chromosomes, and a number of other chromosomal aberrations. These were unequivocally ascribed to streptomycin, demonstrating that the antibiotic could induce genic recombination, translocation and polyploidy. Similarly, Berliner³⁴ has reported that the exposure of basidia of *Gymnosporangium clavipes* to streptomycin caused scattering

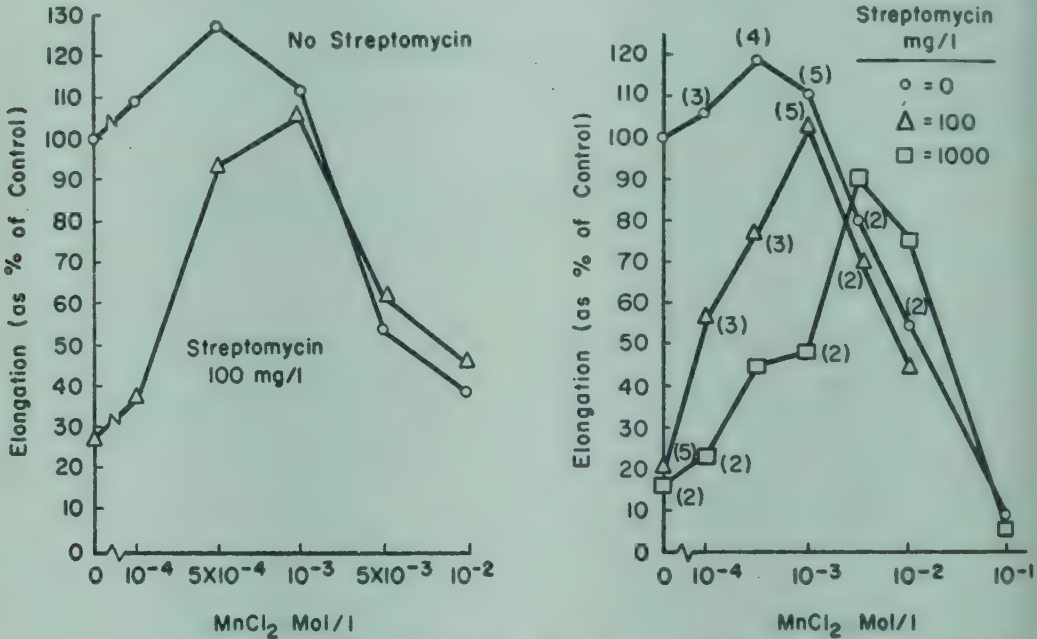


FIGURE 4-8. EFFECT OF THE MANGANOUS ION ON STREPTOMYCIN INJURY. *Left*: Effects of manganous ion concentration on elongation of *Avena* coleoptile sections in presence and absence of streptomycin. *Right*: Effect of manganous ion concentration on elongation of *Avena* coleoptile sections in presence of 2 concentrations of streptomycin.

of chromatic fragments throughout the basidium. This appeared to be due to impaired synchronization between nucleus division and cell wall formation.

It is apparent now that the effects of streptomycin on plant cells are exceedingly profound, disturbing perhaps both their biochemical processes and their genetic apparatus.

A number of reports have been made to the effect that the phytotoxicity of streptomycin could be prevented, or at least moderated, by the presence of the manganous ion (Mn^{++}). Evidence of this action was first presented by Rosen,³⁰⁹ who showed that Mn^{++} (10^{-3} M) could prevent the inhibitory effect on the growth of *Avena* coleoptiles by 100 mcg/ml of

streptomycin (see Fig. 4-8). Pretreatment with Mn^{++} had only a slight positive effect, whereas post-treatments were ineffective. The latter finding substantiates the apparent irreversibility of streptomycin injury. Rosen also noted that more Mn^{++} had to be used as the streptomycin concentration was increased, which suggested competition between the two substances. Some doubt was expressed by Rosen of the validity of this theory, because of the low Mn^{++} /streptomycin mole ratio. However, even on an ionic basis the ratio of Mn^{++} /streptomycin is almost 2:1. That Mn^{++} could reverse to some extent the inhibition of root growth caused by streptomycin was also reported by Gray.¹⁵³ He found that a .0005 M or .001 M solution of $MnCl_2$ was needed for maximum effectiveness in reversing the toxicity caused by 20-40 mcg/ml of streptomycin. Kaufman and Chamberlain¹⁹⁸ showed that 0.3% manganese sulfate added to a 250 mcg/ml spray prevented foliar chlorosis of soybeans. An unsuccessful attempt to suppress this phytotoxicity with manganese was reported by Alcorn and Ark.⁶ They used a maximum of 223 mcg/ml of $MnCl_2 \cdot 4H_2O$ to reverse the effect of a 4-hour stem-dip of carnation cuttings in 10,000 mcg/ml of streptomycin. It would seem that the mole ratio of Mn^{++} /streptomycin was extremely low in this instance.

To explain this phenomenon, one might consider some additional data presented by both Rosen³⁰⁹ and Gray.¹⁵³ They noted in their reports that Mn^{++} was superior to all other cations and to a number of organic compounds such as sugars, amino acids, organic acids, vitamins, etc., in preventing the streptomycin phytotoxicity. Of the other agents tested, only calcium (Ca^{++}) was found to be effective, and it only partially so.

Three facts seem to have considerable bearing on the mechanisms responsible for this Mn^{++} -streptomycin interaction.

1. The manganese concentration must be increased as streptomycin is increased in order to effect a suppression of phytotoxicity symptoms.
2. Mn^{++} appears to be specific in preventing the toxicity, with the Ca^{++} ion being the only other ion that is even partially effective.
3. A comparatively low mole ratio of Mn^{++} to streptomycin can accomplish the suppression.

These facts suggest not only that there is a competition between the two ions for sites on the absorbing surface, but also that both ions may require the same sites for absorption. Specificity of absorbing sites for certain ions and competition between two ions for these sites has been demonstrated by Epstein and Hagen.¹¹⁷

Another attractive speculation regarding the mechanism responsible for the Mn^{++} -streptomycin interaction is that it may be linked to the

relationship of Mn^{++} to chlorophyll synthesis. It has been observed that the chlorosis which develops from exposures to streptomycin is more like a manganese deficiency than the chlorosis associated with nitrogen, iron and magnesium deficiencies.

2. CYCLOHEXIMIDE

It has already been noted that the leaves of some rose varieties become chlorotic when sprayed with cycloheximide at 0.1–0.2 mcg/ml.³²⁷ According to Hawthorne and Wilson,¹⁷¹ who studied the cytological effects of this antibiotic on onion root tips, cycloheximide, like streptomycin, is a mutagenic agent. The chemical is capable of inducing mitotic deviates which are incapable of further division. These workers were also of the opinion that cycloheximide prevented the normal organization (development) of tissue, rather than the destruction of structures already formed. These results may explain the observations made by Cation,⁶⁰ to the effect that an early application of cycloheximide resulted in some fruit dwarfing and that 2–10 mcg/ml applied during the bloom resulted in total fruit abscission.

The literature discloses, nevertheless, an extremely wide range of cycloheximide concentrations which are toxic to plants. Gottlieb *et al*¹⁴⁸ evaluated the sensitivity of tomato, bean, geranium, peach and strawberry foliage to 1, 10, 100, and 1,000 mcg/ml of this antibiotic. Tomato and bean were most sensitive, showing necrotic spots at 1 mcg/ml. Peach foliage was not injured by 10 mcg/ml, but trees were completely defoliated by 100 and 1,000 mcg/ml. Strawberry, on the other hand, was insensitive to sprays at 1,000 mcg/ml. Vaughn and his associates³³⁰ observed that the germination of wheat, radish, and bean seeds was severely inhibited by 100 mcg/ml of the antibiotic. They also noted that pea seeds took up less water, and were, as a result, less severely inhibited. Using a 4-hour exposure to the antibiotic solution, these workers observed that a delay and/or inhibition of germination was proportional to concentration. In additional experiments, they found roses sensitive to 2.5 mcg/ml. Wallen and co-workers³⁴⁵ also investigated the effect of this antibiotic on pea seeds and detected an inhibitory effect at 1 mcg/ml. Calla rhizomes were treated by Dosdall¹⁰¹ with 5 mcg/ml for one hour and an extreme toxicity was found. The cycloheximide analogues, e.g. semicarbazone and oxime, were studied by Hacker and Vaughn,^{165,166} who found them less toxic than the parent molecule. Spring wheat showed slight to moderate phytotoxicity when sprayed with 200 mcg/ml of the semicarbazone, and slight toxicity when sprayed with 50 mcg/ml of the oxime. Hamilton and Szkolnik¹⁶⁸ reported that injury encountered

with the cycloheximide derivatives at 10 mcg/ml or lesser concentrations was of no significance in greenhouse tests, but ranged from moderate to severe at 60 mcg/ml.

3. GRISEOFULVIN

This antibiotic is beginning to find increasing commercial application as an antifungal compound. Compared with the two materials previously discussed, it is much less phytotoxic.

Brian and his group,⁴⁶ who have done most of the experimental work with this antibiotic, have reported slight root and shoot stunting of lettuce seedlings grown for 4 weeks in 10 and 50 mcg/ml solutions. Oat seedlings placed for 15 days in similar concentrations demonstrated some stunting and some foliar tip burn 3–4 weeks after treatment. Stokes³²³ showed that wheat seedlings grown in solutions of 5 mcg/ml or higher concentration were swollen just behind the root tip. Similarly, fungal hyphae in contact with griseofulvin showed swellings or bulbous excrescences. These areas have been observed to rupture, expelling protoplasm into the ambient solution. This action suggests that the phytotoxicity observed may be due to disorganization of the cell wall and the plasma membrane. Below 5 mcg/ml, no phytotoxic effects seem to occur. Some inhibition of seed germination has been reported by Wright,³⁶² and Rich observed that the antibiotic applied at the rate of 40 mcg/kg. of soil had a slight stunting effect upon lettuce.

4. POLYMYXIN

This antibiotic does not appear to hold much promise as an antimicrobial agent in plant pathology and, as a result, its experimental use has also been limited. Nevertheless, one of the accounts of phytotoxicity induced by this compound merits special consideration. Norman²⁷³ observed this negatively-charged polypeptide antibiotic to have adverse effects on growth and function of barley roots. He found that 5 mcg/ml of polymyxin depressed the growth of roots (which require calcium), when the Ca^{++} concentration was 10^{-5} or 10^{-4} . However, at 10^{-3} the depression was less, and at 10^{-2} it was annulled. He calculated that the inhibitory effect of 11.25 mg of polymyxin could be offset by 300 mg of Ca^{++} and, curiously, neither Na^{+} or K^{+} ions could minimize this injury. Brief exposures of roots to 50–100 mcg/ml of polymyxin resulted in cessation of growth and a weight loss due to leakage of organic and inorganic cellular constituents. The effect of polymyxin seems to be due to its surface-active qualities, causing a disorganization of the cellular membranes and thereby a change in their permeability. It is apparent

therefore why the deleterious effects of polymyxin could not be reversed by additions of Ca^{++} once the injury had occurred.

The suppression of polymyxin injury by Ca^{++} may be due to the fact that, like the manganese-streptomycin interaction, both Ca^{++} and polymyxin are retained at the same absorption sites. The large excess of ions needed to effect this suppression may be accounted for by the differences in molecular size and the number of electrical charges (reactive sites) which each molecule carries.

Barton and McNab^{30a} have substantiated the phytotoxic level discussed by Norman. They noted that the growth of wheat roots was significantly suppressed at concentrations above 3.2 mcg/ml.

5. TETRACYCLINES

Competitive absorption has now been discussed for two antibiotics, and according to Barton and McNab,^{30a} oxytetracycline injury to roots can be mediated by calcium, but not by magnesium. In additional experiments, sprays containing concentrations of oxytetracycline up to 500 mcg/ml were applied to foliage of cabbage, tomato, carrot and snapdragon, and a slight chlorotic effect was observed at the highest concentration.

Infiltration of peach trees by oxytetracycline was investigated by Dunegan;¹⁰³ when 1.7 grams of the antibiotic dissolved in 28.4 liters of water were absorbed, a yellow-green leaf mottle (chlorosis) was observed. This effect has been noted by the writer when sprays of oxytetracycline at 100 mcg/ml were applied to 1-year-old peach buddlings in the nursery row, and it appears to be a characteristic phytotoxic response of the genus *Prunus* to the tetracyclines.

Pramer and Wright²⁸⁷ evaluated the sensitivity of mustard, red clover, cucumber and wheat seeds to oxytetracycline, chlortetracycline and three other actinomycete antibiotics, chloramphenicol, neomycin and streptomycin. Chloramphenicol, chlortetracycline and tetracycline were more toxic to seeds than were the remaining two antibiotics. These three antibiotics suppressed chlorophyll synthesis at lower concentrations than did either streptomycin or neomycin. In this study chloramphenicol was also observed to inhibit the formation of anthocyanin in the cotyledons of mustard.

6. OTHER ANTIBIOTICS

A number of other antibiotics have been observed to cause phytotoxic effects. Among these are alternaric acid,^{280,362} glutinosin, mycophenolic acid, and gliotoxin.³⁶² Leben and Keitt^{218,223} have reported that helixin inhibited germination of tomato seeds at 25 mcg/ml and that antimycin

demonstrated a specific inhibition of embryonic tomato tissue. In fact, the injury from this compound appeared limited to meristems and, as a result, these investigators suggest that the effect may be due to an inhibition of the cytochrome oxidase system.

Leben and Keitt²²³ state that, while a good deal of attention has been given to antibiotics for plant disease control purposes, very little has been paid to their inhibitory effects upon plants *per se*. In fact, they contend that most of the phytotoxic effects observed in disease control work remain unpublished. They further state that, "antibiotics are a group of highly selective and potent materials that merit more study because of their injurious effects on plants. Materials of much practical significance might thus be uncovered." The author submits that a closer inspection of these phytotoxic effects might also offer some clues as to the mode of action of these compounds.

H. SYSTEMICITY OF ANTIBIOTICS

1. ABSORPTION

It was rather unexpected that the comparatively large antibiotic molecules could be absorbed and translocated by plant cells. Successful *in vivo* experiments eradicating artificial infection following foliar sprays, stem soaks, trunk infusions and other methods of application furnished indirect proof that a number of antibiotics were systemic in their action. There is little doubt now that some antibiotics may be considered chemotherapeutants in the strictest sense of the word. Although most of these compounds have been successful as systemic protectants, some have been shown to be systemic eradicanants as well.

The degree to which these antibiotics are systemic depends primarily upon the method of application, the plant part exposed, the length of exposure, the concentration and the specific antibiotic used. It is incorrect, therefore, to describe streptomycin categorically as a locally systemic compound or as a material with limited systemic activity. Although the systemicity of streptomycin may be less pronounced when it is applied as a foliar spray than when it is used as a foliar or stem immersion, it is nevertheless a systemic compound.

Theoretically at least, substances may enter plant cells and tissues by diffusion, by the Donnan equilibrium mechanism and metabolically (actively) by the expenditure of respiratory energy. Accumulation of solute against a concentration gradient is impossible by diffusion alone. A comparatively slight accumulation can be realized through a Donnan equilibrium condition; however, significant accumulation implies active

uptake and the expenditure of energy. It is well known that nonionic compounds enter plant cells by diffusion, with the internal or cellular concentration finally attained at equilibrium being equal to the concentration of the ambient solution. On the other hand, ionic or dissociated substances may be actively absorbed against a concentration gradient.

Whether one considers the absorbing surface of plant cells to be the rather restricted cell wall surface, or to have the depth associated with Briggs and Robertson's^{50a} "free space" and Epstein and Hagen's¹¹ "outer space," absorption is profoundly influenced by the electrical charge carried by the solute.

a. Antibiotic Absorption at the Cellular Level

Pramer²⁸⁶ has performed a number of experiments that suggest rather strongly that streptomycin is accumulated in plant cells against a concentration gradient. Using the large-celled fresh water algae *Nitella clavata*, he demonstrated that cells growing in a 25 mcg/ml streptomycin solution contained the antibiotic in their vacuolar fluid at a concentration of more than 175 mcg/ml after 18.5 hours. In similar experiments, the amount of chloramphenicol within the cell was less than half the concentration of the external solution, suggesting that this neutral antibiotic was absorbed by diffusion. Paradoxically, penicillin, which is an acidic compound, and might have been expected to be absorbed

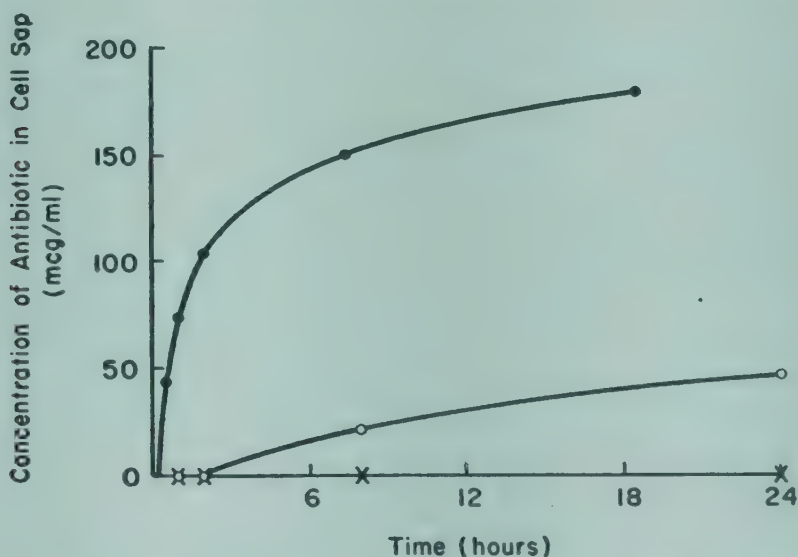


FIGURE 4-9. STREPTOMYCIN ABSORPTION BY *N. clavata*. The absorption of antibiotics by cells of *N. clavata*. Cells were suspended in the following antibiotic solutions prepared with M/45 phosphate buffer of pH 5.1: ●, 25 mcg/ml of streptomycin sulfate; ○, 136 mcg/ml of chloramphenicol; and ×, 128 mcg/ml of potassium penicillin G.

actively, as was the basic streptomycin, gave no evidence of being absorbed at all (see Fig. 4-9).

Further evidence was presented by Pramer²⁸⁸ to the effect that streptomycin is actively absorbed, since the process could be inhibited by enzyme poisons (see Table 4-7). It was also suggested that specific

TABLE 4-7
INFLUENCE OF RESPIRATORY INHIBITORS ON
STREPTOMYCIN UPTAKE

Inhibitor	Concentration M	Streptomycin uptake % of control
NaAsO ₂	10 ⁻²	100
o-Phenanthroline	10 ⁻²	100
NaF	10 ⁻²	72
NaN ₃	10 ⁻²	33
Indoacetic acid	10 ⁻²	7
Na ₂ EDTA	10 ⁻²	3
2,4-DNP	10 ⁻²	5
2,4-DNP	10 ⁻³	16
2,4-DNP	10 ⁻⁴	32
2,4-DNP	10 ⁻⁵	100

(After Pramer²⁸⁸.)

sites of limited capacity are involved in the absorption of this antibiotic, since the divalent cations Ca⁺⁺ and Mg⁺⁺ interfered with streptomycin absorption. These data are somewhat analogous to those showing the complete reversal of streptomycin toxicity by Mn⁺⁺ and at least partial suppression by Ca⁺⁺.^{153,309}

The most recent experiments in this series by Litwack and Pramer²²⁸ show that streptomycin absorption increases as the antibiotic concentration in the ambient solution increases (see Figure 4-10). Further, the rate of uptake fits the Michaelis-Menton equation, producing values of $.05 \times 10^{-4}$ to 3×10^{-4} for K_s , and the energy of activation for this uptake was calculated to be 7,512 cal/mole. (This is 3,000 cal/mole in excess of that required for free diffusion.) These data suggest that the process is mediated by an ion-binding carrier system requiring an expenditure of energy by the plant cell. The scheme for this system is presented below:



Thus, the neutral complex SC migrates across the membrane and is released at the inner surface of the diffusion barrier (tonoplast).

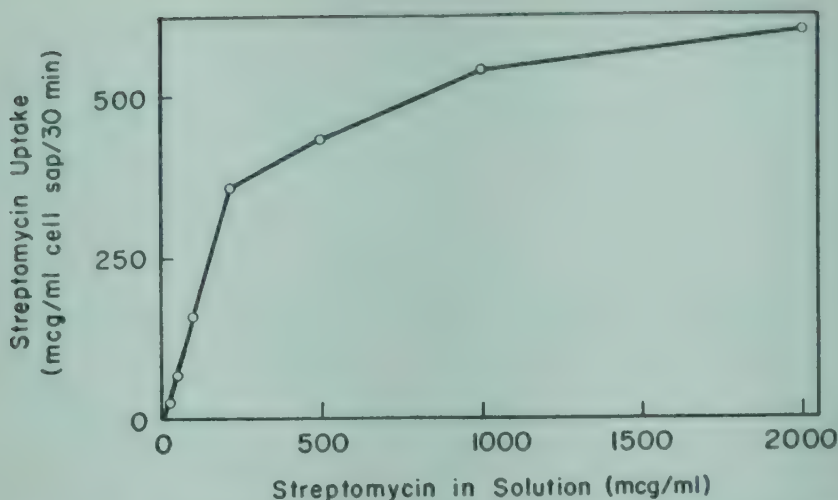


FIGURE 4-10. INFLUENCE OF CONCENTRATION ON STREPTOMYCIN ABSORPTION. The influence of concentration on the rate of streptomycin uptake. Cells were treated with various amounts of streptomycin in 25 ml. of 0.022 M phosphate buffer at pH 6.0.

b. Antibiotic Absorption by Plant Tissues and Organs

Studies of the kinetics of antibiotic absorption have been conducted in detail at the cellular level only for streptomycin. Nevertheless, much can be deduced from antibiotic absorption experiments involving plant tissues and organs. Further, it is not at all certain that results obtained with *Nitella* are applicable to higher plants for, as is shown below, even the results between species are often conflicting.

1. *Absorption by Seeds:* In experiments to control the seed-borne fungus *Ascochyta pisi*, Dekker^{94,95} and Oort and Dekker²⁷⁵ revealed data that suggest significant absorption of rimocidin, and particularly pimaricin. Pea seeds soaked for 24 hours in 100 mcg/ml of pimaricin contained 50–75 mcg/ml in the embryo and seed coat, 24–40 mcg/ml in the outer parts of the cotyledons and 5 mcg/ml in the interior of the seeds. Mirzabekian and Menkova²⁵¹ have described the absorption of penicillin, streptomycin, grizemin and “antibiotic 103” by tomato and cabbage seeds after a 24-hour dip in 1,000 antibacterial units.* Evidence has been presented by Rangaswami²⁹⁷ to the effect that the mycothricin complex appears to diffuse into wheat seeds.

2. *Absorption by Stem Tissue:* The classical examples of antibiotic absorption via stem tissue are those of Mitchell, Zaumeyer and their associates.^{252,253,254} In their experiments a streptomycin-lanolin-Tween 20 paste was applied to the bean stem-internode below the primary leaves

* This unit is apparently based on the activity of the antibiotic against *Corynebacterium michiganense*.

Absorption was demonstrated by successful control of artificial inoculations with *P. phaseolicola* and by bioassays.

It has also been shown that antibiotics may be absorbed through tree trunks. Dunegan and Wilson^{103,104} demonstrated the uptake of oxytetracycline in this fashion using an infusion technique. Ark and Alcorn²³ and Goodman and Johnston¹⁴³ revealed streptomycin absorption from antibiotic-containing capsules placed in bore-holes in pear and apple tree trunks. Krasilnikov,²⁰⁹ apparently using a similar technique, reported penicillin to be taken up more rapidly than streptomycin, and described a species variation in extent of absorption. For example, many rosaceous species absorbed both antibiotics, whereas elm, ash, acacia and lime were unable to absorb any. In addition, it was noted that some species appeared to inactivate the two antibiotics. One gram of birch tissue could absorb 18,000 units of penicillin and could completely inactivate 6,000 units; in similar experiments with streptomycin, it absorbed 6,000 units and inactivated 3000 units. Ion adsorption might explain the inactivation of streptomycin, but it does not account for the reduction in penicillin activity. Pratt and Dufrenoy^{289a} have stated that the fundamental course of deterioration of penicillin is by hydrolysis and that water is the worst enemy of penicillin. It is also known that an extremely small amount of phosphate extends the antibacterial potency of penicillin. Perhaps this quantity was not available in the birch tissue homogenate, resulting, therefore, in a rapid hydrolysis of penicillin.

Petiolar (leaf-stem) uptake of antibiotics by cherry laurel was studied by Charles,⁵⁹ using both streptomycin and penicillin. In preliminary experiments with acidic (acid fuchsin) and basic (methylene blue and safranin) dyes, he noted that the basic dyes were confined to the petiole butts, whereas the acid fuchsin reached the leaf extremes in two to three hours. Analogously, acidic penicillin entered the leaf in 45 minutes, while the basic streptomycin could not be detected after 4 hours. It is apparent that these results are not in good agreement with the data obtained from experiments with *Nitella*.^{286,288} Charles suggests that the xylem elements are negatively charged with OH⁻ ions derived from ionized water, and in this way the non-ionized lignin and cellulose of the xylem can attract the positively-charged streptomycin molecules. Alcorn and Ark^{5,6} investigated the absorption of streptomycin, neomycin and the three tetracycline antibiotics by cuttings of carnation and pyracantha. Generally, the amphoteric tetracyclines were more readily absorbed than the two basic compounds. However, upon the addition of 1% K₂HPO₄ to streptomycin solutions, uptake of this antibiotic was strikingly facilitated. For example, cuttings immersed for 4 hours in streptomycin at 1,000 mcg/ml, with and without K₂HPO₄, showed antibiotic activity

extending 3.0 and 0.3 inches, respectively, from the stem bases. Alcorn and Ark suggested that the monovalent K^+ ion competes with streptomycin for the negatively-charged sites on the xylem walls. In experiments conducted by the writer on antibiotic absorption by carnation cuttings, it was observed that neither the amphoteric dye congo red nor streptomycin was absorbed. However, the results of Alcorn and Ark were duplicated for these two compounds when K_2HPO_4 in 1% concentration was added to the solutions. Furthermore, this improved uptake of streptomycin and congo red was obtained over a pH range of 5-8 (see Fig. 4-11).



FIGURE 4-11. THE INFLUENCE OF DIPOTASSIUM PHOSPHATE ON THE ABSORPTION OF STREPTOMYCIN. Triplicate carnation stem slices taken 3 (lower) and 6 (upper) millimeters above point of immersion. Carnation cuttings were immersed for 24 hours in streptomycin at 200 mcg/ml with K_2HPO_4 at 1 percent (plate at left) and without (plate at right).

The data on absorption of antibiotics by cuttings become slightly more difficult to evaluate when one considers the results presented by Robinson and co-workers³⁰⁷ regarding antibiotic absorption by chrysanthemum cuttings. Their studies included streptomycin and oxytetracycline and indicate that both antibiotics are readily absorbed. In fact, streptomycin was detected in the guttation water of leaves from some cuttings.

Rudolph³¹⁰ placed the basal ends of pear shoots into penicillin solutions and allowed absorption to take place under a low vacuum. Sap from the distal ends of the absorbing shoots showed a degree of antibiotic activity, corrected for dilution by fluids in the xylem vessels prior to treatment, equal to that of the dipping solution. In a similar experiment

by Brown and Heep,⁵⁵ relatively woody plum shoots, known to be infected with *X. pruni*, were infused overnight with streptomycin under a negative pressure. Although a bioassay was not performed, indirect evidence of absorption was revealed when attempts to isolate the pathogen from treated wood failed. Mirzabekian²⁴⁹ reported that peach and apricot cuttings took up streptomycin, grizemin and antibiotic "number 6."

In a study by Pramer²⁸⁵ on the absorption of streptomycin and chloramphenicol by tomato and broad beans, it was disclosed that, regardless of species, antibiotic absorption was more effective through cut shoot bases than through plant roots. In addition, the two antibiotics were more readily absorbed by tomato than broad bean; in fact, streptomycin was not detected in tissues of rooted broad bean plants.

3. *Absorption Through Roots*: A number of antibiotics, as stated previously, are adsorbed by the clay and organic matter fractions of soil, thereby precluding soil applications in most instances. However, crops placed in the field as transplants offer an opportunity for making at least one antibiotic application via their root systems.

Perhaps the earliest account of antibiotic absorption through plant roots was that of Anderson and Nienow,¹² who reported that wheat, radish and soybean seedlings absorbed streptomycin through their root systems. Bioassays of soybean expressate made by these investigators yielded 5–10 mcg/ml from plants that had been grown in solutions containing 50 mcg/ml. Mirzabekian and Menkova²⁵¹ reported that a number of antibiotics were absorbed by the roots of tomato, cabbage and tobacco seedlings. Tomato seedlings, 15–20 cm tall, were placed in these antibiotic solutions, and their uppermost leaves were assayed at 30-minute intervals. Penicillin was detected after 30 minutes, streptomycin and grizemin in 6 hours, while chlortetracycline and globisporin appeared after 8 and 16 hours, respectively. Similar data were obtained from cabbage and tobacco seedlings. Klemmer and co-workers²⁰⁶ demonstrated that root systems of tomato plants absorbed 150 mcg/ml of chloramphenicol and 50 mcg/ml of oxytetracycline from dipping solutions of 250 and 125 mcg/ml, respectively.

Crowdy and his associates⁸² have shown that the roots of broad beans absorb both chloramphenicol and griseofulvin. It should be recalled, however, that Pramer²⁸⁵ found streptomycin to be absorbed slowly by cuttings and not at all (in 18 hours) by rooted plants of this species. A similar observation was reported by Dye¹¹¹ concerning the absorption of streptomycin by cut stems and rooted peach seedlings (see Table 4–8). Crowdy⁸⁵ has attributed this observed lag in streptomycin uptake to the necessity for saturating all adsorbing sites before absorption could

TABLE 4-8
UPTAKE OF STREPTOMYCIN THROUGH CUT STEMS
AND ROOTS OF PEACH SEEDLINGS

Time in solutions (hr)	Stem diameter (mm)	Streptomycin concentration, mcg/ml					
		Check		10 Zone dia., (mm)*		100	
		Cut stems	Roots	Cut stems	Roots	Cut stems	Roots
1	2-3	—	—	9.4	—	15.3	—
6	2-3	—	—	11.0	—	16.3	4.6†
24	2-3	—	—	14.1	trace	21.3	12.3

* Mean zone diameter from six disks removed from stems 4-9 in. above solutions.
† Lower stem region only. (After Dye¹¹¹.)

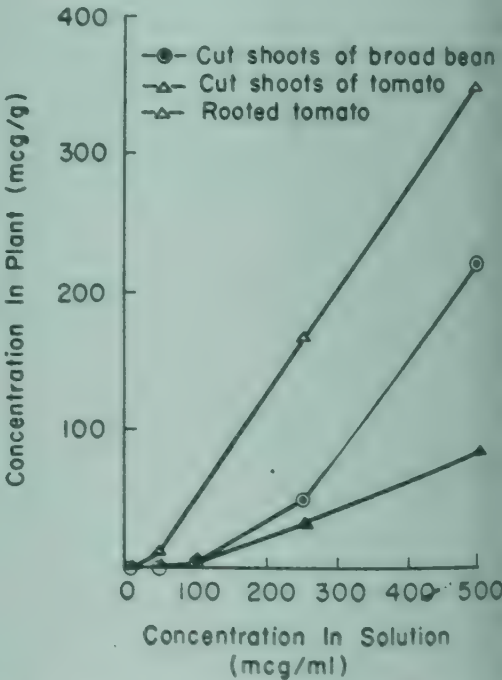
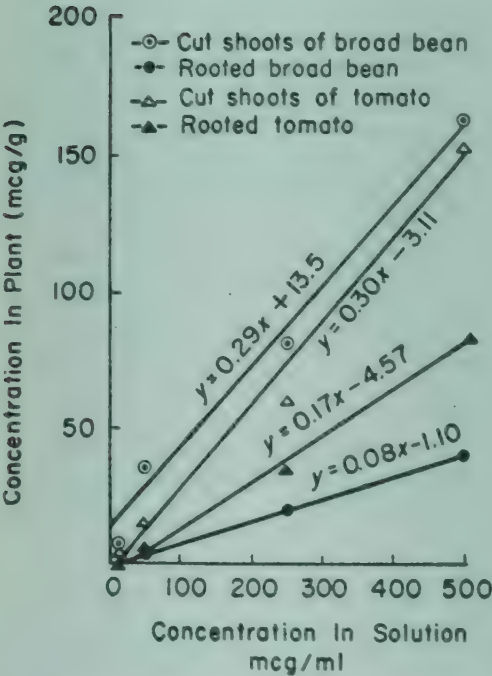


FIGURE 4-12. CHLORAMPHENICOL AND STREPTOMYCIN UPTAKE BY CUT SHOOT AND ROOT SURFACES. Left: Relationship between the concentration of chloramphenicol in solution used for treatment and the concentration in the plants. Right: Relationship between the concentration of streptomycin in the solution used for treatment and the concentration in the plants.

progress. In support of this viewpoint, one need only compare the specific surface presented by a bean plant root system and by the base of a bean stem. Furthermore, it was apparent from Pramer's²⁸⁵ experiments that the lag was more pronounced at lower concentrations (see Fig. 4-12).

Additional experiments by Brian and his group⁴⁶ and by Stokes³²³ indicate that the neutral griseofulvin molecule is readily absorbed by roots of a number of plant species. The antibiotic appears rapidly and in significant quantity in all tissues, and frequently in guttation water.

Parmer²⁸⁴ treated cucumber seedlings in 50 mcg/ml solutions of streptomycin, chloramphenicol, oxytetracycline, chlortetracycline, and neomycin. Streptomycin was detected in cotyledons and leaves at a concentration of 25 mcg/ml, whereas the tetracyclines and neomycin could not be detected. The variation in concentration between chloramphenicol and streptomycin suggests different mechanisms of absorption for each, with streptomycin being actively absorbed and chloramphenicol entering through simple diffusion. It is difficult to explain the apparent failure of the tetracyclines to enter the cucumber seedlings, unless the absorbing surfaces of different plant species vary considerably in their degree of selectivity and permeability. Active absorption of streptomycin by peach seedlings has also been reported by Dye.¹¹¹ Despite the negative charge carried by neomycin, this antibiotic probably does not enter plant cells without damaging, or at least altering, cellular permeability. Norman²⁷³ has ascribed this phytotoxicity, as was stated earlier in this chapter, to the strong surfactant effect which this compound displays.

The consistency with which the data on antibiotic absorption remain inconsistent is shown by the experiments of Blanchard and Diller.³⁵ Lima bean seedlings grown in chlortetracycline concentrations of 100 and 1,000 mcg/ml contained the antibiotic in root tissue at concentrations of 115-230 and 1,500-5,820 mcg/ml. Since these roots were washed prior to assay, accumulation of this amphoteric antibiotic against a concentration gradient is indicated. Of additional interest in these experiments is the fact that despite the high concentrations encountered in root tissue, only 1.1-7.7 mcg/ml were found in the leaves, suggesting a binding or complexing situation in the root regions.

The absorption of cycloheximide by roots of wheat seedlings grown in quartz and sand has been reported by Wallen and Millar.³⁴⁸ Similarly, Lemin and Magee²²⁵ observed labeled cycloheximide-acetate, 2-C¹⁴, to be absorbed by the roots of tomato plants.

4. *Absorption Through Leaves:* Antibiotics are applied to foliage primarily as sprays and, less frequently, as dusts. Assuming ideal

conditions for applying antibiotics by either method, two factors which influence absorption critically are the concentration applied and the amount deposited. It is apparent that where antibiotic absorption has been observed, the quantity of antibiotic absorbed increases as the concentration applied increases. However, with effective exposure time (period of absorption), limited in the case of sprays by drying and "run off," it is obvious that the ratio of amount deposited to the amount applied should be a narrow one. According to Horsfall,¹⁸⁹ the electrokinetic charge of the toxicant is an extremely important factor in deciding the value of the above ratio. Negative charges are carried at the leaf surface, which attract positively-charged molecules and repel negatively-charged molecules. In this way Horsfall explains the observation that positively-charged molecules are deposited in proportion to the logarithm of the concentration, whereas those carrying a negative charge are deposited in proportion to the concentration. Horsfall has also stated that as dust particles leave the dusting machine, they receive a negative electrostatic charge which curtails deposition at the negatively-charged leaf surface. This phenomenon may explain the apparent superiority of antibiotic spray formulations over antibiotic-impregnated dusts.

Evidence that antibiotics are absorbed by leaves is of two types: direct and indirect, the former being substantiated by bioassays or by quantitative chemical measurements, and the latter by the suppression *in vivo*, of an artificially administered pathogen.

Bioassays have demonstrated the absorption of streptomycin by foliage of peaches,^{86,111,113} beans,^{144,145,153,154,155,159} tomatoes and tobacco.¹⁵ Indirect evidence for the foliar absorption of streptomycin has been reported for apples,^{135,136,137,174,255,359} beans,^{252,253,254,261} peaches^{111,113,108} and broccoli.³⁶⁴ Other antibiotics that have been found to be absorbed significantly by foliage, either directly or indirectly, are oxytetracycline,^{135,136,153,359} chlortetracycline,¹⁵³ cycloheximide,^{167,168,348} and griseofulvin.⁸

A disparity in absorption, similar to the difference between cut stems and plant roots, has been observed for upper and lower leaf surfaces. Dowler and Goodman^{101b} painted the upper and lower surfaces of coleus leaves with streptomycin and noted that slightly more antibiotic was absorbed through the lower surface (see Fig. 4-13). Davis and Rothrock³ showed that griseofulvin decreased the per cent of leaf area infected by *A. solani* more significantly when sprays were applied to the lower surfaces of tomato leaves and inoculated on the upper surfaces than when these procedures were reversed. Similarly Zaumeyer³⁷³ reported that treating lower surfaces of bean leaves with either oligomycin or anisomycin provided better protection against infection by *Uromyces phaseoli* var. *typica* than did treating upper surfaces. In these experiments the un-

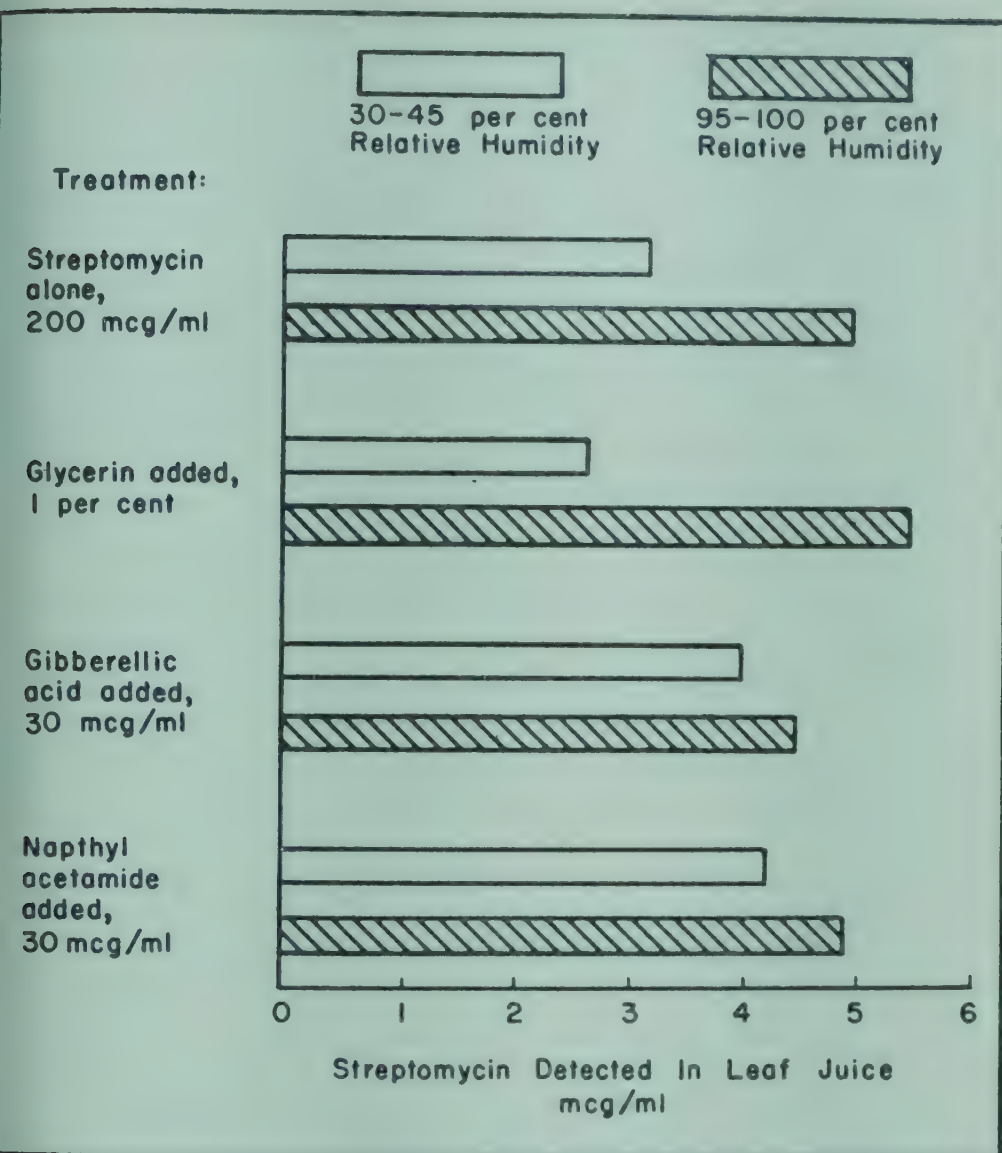


FIGURE 4-13. ABSORPTION OF STREPTOMYCIN BY *Coleus* FOLIAGE. The effect of relative humidity on the absorption of streptomycin by *Coleus* from solutions with and without additives.

reated surface was inoculated 24 hours after the antibiotic was applied. Excellent protection against upper leaf surface inoculation with *Colletotrichum lindemuthianum* resulted from treatment of lower surfaces with streptomycin, whereas an upper surface antibiotic application was ineffective.

It was observed in both series of experiments^{89,373} that disease symptoms were more severe in leaves of control plants when the inoculum was applied to the lower surface.

From these data one might surmise that the observed effects reflect in some way the distribution of stomates on the two leaf surfaces. Stomates occur in both the upper and lower epidermis of most plant species; however, they are commonly more numerous on the lower epidermis. There are also some species in which stomatal openings are confined to the lower epidermis, and a few hydrophytes whose stomata are limited to the upper surface.

In coleus leaves the stomates are located in the lower epidermis only, there being some 14,000 openings per cm^2 . It has been calculated that the number of stomatal openings for upper and lower surfaces is 4,000 and 28,000/ cm^2 for bean leaves, and 1,200 and 13,000/ cm^2 for tomato foliage. These figures are certainly suggestive of a stomatal influence on antibiotic absorption. However, this influence may be more apparent than real.

The transpiration of a leaf depends upon the difference between the vapor pressure of the water in the leaf and the vapor pressure of the water in the atmosphere. Since the vapor pressure of the water in the leaf is usually the greater, a gradient favoring movement of moisture from the plant to the air is established. Thus, migration of spray material through the stomatal openings during the day would at best be slow. At night when the gradient usually decreases and an equilibrium between the vapor pressures of atmospheric and plant moisture is possible, the stomata are generally closed.

Other modes of entry might be postulated; for example, Morgan *et al*²⁵⁵ reported antibiotic activity in apple foliage as high as 38.6 mcg/leaf two days after a 100 mcg/ml spray with Agrimycin.* This relatively high internal concentration indicates a more favorable, or at least a less restrictive, pathway for the entry of antibiotics than through the stomata.

Direct penetration of the thick cuticular layer of the leaf epidermis is suggested as a possibility, and this possibility is supported by the absorption of streptomycin through the upper epidermis of coleus noted by Dowler and Goodman^{101b} (see Fig. 4-13).

A closer look at the barrier presented by the walls of epidermal cells is possible through a description of the arrangement of the chemical components of the wall. According to Frey-Wyssling¹²² these components are as follows: the hydrophylic lamellae consisting of cellulose and pectins, the layers of wax molecules in radial arrangement, and between the two in random orientation, the amorphous polar cutin. The cellulose and pectins are located predominantly in the inner regions of the wall, whereas the waxes are the most numerous towards the outer surface.

* The trade name of Chas. Pfizer and Co., Inc. for an antibiotic formulation which contains 15 per cent streptomycin and 1½ per cent oxytetracycline.

Since the cutin contains both hydrophylic (OH, COOH) groups and hydrophobic (CH₃) groups, the former are oriented towards the cellulose and the latter towards the wax. This arrangement provides a convenient pathway through which water molecules may traverse an ostensibly watertight layer. In fact, as a result of its hydrophylic groups, the cutin is capable of swelling and, due to its random orientation through the waxy phase of the cell wall, might well permit the penetration of water-soluble substances.

It is possible that the effect of glycerol and other humectants^{144,145,158} in increasing the absorption of streptomycin is actually an hydration of the cutin. The swelled cutin could then force the wax lamellae apart, giving the streptomycin molecules easier access to the hydrophylic cellulose and pectin fraction of the cell wall.

The degree to which the cell wall becomes hydrated would, of course, depend upon how long the leaf surface remained wet. Zuekel *et al.*,³⁷⁶ working with a growth regulator, maleic hydrazine (MH), noted that the relative humidity was the greatest single factor affecting the absorption rate. Temperature at controlled humidities had less of an effect, and surfactants were generally non-effective in improving the absorption of MH. Somewhat similar results were reported by Koontz and Bid-dulph,²⁰⁸ while studying the uptake of radioactive P³² applied as NaH₂P*O₄. It was observed that glycerin increased the absorption of P³²; however, surface active agents (nonionic, anionic, and cationic) were ineffective in increasing absorption. This was believed due to the uniform spreading and concomitant rapid drying, since it was observed that surfactants at times increased the drying time threefold. This ineffectiveness of surface-active additives has also been reported by Gray.¹⁵⁸

Improved hydration of the cell wall may explain the movement of water-soluble substances through the upper epidermis, but it does not account for the greater absorption of antibiotics through the lower epidermis. The morphology of lower leaf surfaces offers some clues and brings into consideration the leaf hairs or trichomes. Although cuticular transpiration has been calculated to be only 1/20–1/40 that of stomatal transpiration, the presence or absence of trichomes profoundly influences this transpiration. Since water vapor can pass through these structures in an outward direction, inward movement may be assumed as well. In many plant species the trichomes are exceedingly numerous, providing leaves with greater specific surface on their undersides. This additional absorbing surface could account for the increases in antibiotic uptake that have been reported.

Protuberant veins which are classically demonstrated by the under-

surfaces of coleus leaves may also affect foliar absorption. It is obvious that they will at least increase specific surface, and thereby increase absorption. Perhaps even more significant is the direct absorption into the veins which may occur according to Van Overbeek.^{337a} He has stated that when the epidermis is stripped from the leaf, water will wet the walls of the underlying mesophyll cells. Pure water, on the other hand, adheres to the surface of a vein and is adsorbed into it. According to Esau,¹¹⁸ the surface of a vein is composed of a specialized parenchymatous tissue composed of thin-walled cells, the bundle sheath cells. Cells similar to those of the bundle sheath frequently extend to the upper and lower leaf epidermis and are concerned with conduction. Thus aqueous solutions applied to the epidermal cells of veins may be absorbed rapidly, with a minimum of dilution, into the conduction elements of the vein itself. Dunegan and Wilson,¹⁰⁷ using a thread-wick injection procedure, punctured apple and pear leaves adjacent to large veins.* As a result of this infusion, not only did the mesophyll adjacent to the puncture become chlorotic, but the veins on that half of the leaf blade were affected similarly.

There seems to be little doubt that the roots of some plants absorb griseofulvin actively,^{46, 82, 323} and that streptomycin is absorbed by cells of *Nitella* through an expenditure of respiratory energy. Nevertheless, the absorption of antibiotics applied to foliage as a spray, as an immersion or in any other fashion has not been shown, to the knowledge of the author, to be metabolically controlled.

A possible mechanism responsible for the improved absorption by foliage of streptomycin when combined with a humectant has already been advanced. It is more difficult, however, to explain the greater uptake of streptomycin by bean foliage when growth regulators such as naphthyl acetamide (NA) and gibberellic acid (GA) were added to the spray formulation.¹⁴⁵ Yet it is quite possible that these results reflect metabolically mediated absorption, and indirect evidence of this mechanism has been recorded by Dowler and Goodman^{101b} (see Fig. 4-13). From these data it is apparent that where plants sprayed with streptomycin alone, or in combination with 1% glycerin, are maintained in a saturated atmosphere, absorption of the antibiotic is improved. However, it is also apparent that the relative humidities prevailing under the conditions of the above experiments did not influence the absorption obtained when growth regulators were added to the spray. In companion experiments *Coleus* plants kept under clear plastic covers absorbed more streptomycin than plants under black plastic. These results suggest that growth

* One end of the thread-wick was inserted into a vial containing streptomycin, and the other into the vein puncture.

regulators increase streptomycin absorption through an effect on the production of substrate for metabolic activity (photosynthate), and/or an effect upon metabolic rate *per se*.^{101a}

2. TRANSLOCATION

When one considers the movement of absorbed solutes in plants, it is generally conceded that the downward or polar translocation proceeds primarily through the phloem, and that acropetal movement is accomplished via the xylem. It appears also that mineral substances are carried primarily through the xylem vessels, whereas organic compounds move chiefly in the phloem sieve-tubes. Beyond these generalities on phloem and xylem transport, discussion without controversy is practically impossible. Esau *et al*¹¹⁹ cite no less than five hypotheses concerning phloem transport mechanisms.

It has been fairly well established that at least a certain portion of the transport through the phloem is dependent upon respiratory activity. The process is blocked by low temperature, low oxygen tension and certain respiratory poisons. The unknown factors are the energy source and the manner and place in which it is used. On the other hand, the movement of solutes in the xylem takes place under the influence of transpiration, as does the movement of water.

It has been demonstrated by radioactive tracer techniques that lateral movement from the xylem to the phloem occurs readily. Completing the translocatory circuit from the sieve tubes of the phloem to the other living cells, and from cell to cell, is the network of protoplasmic strands, the plasmodesmata. Finally, it is still not clear whether the solute moving in the plasmodesmata enters the vacuole of each cell as it migrates from the point of absorption towards the vascular system, or whether this movement is through the cytoplasm.

There is, of course, considerable evidence for the transport of such diverse and comparatively large organic molecules as dyes, growth regulators, viruses and antibiotics in the vascular system. It would seem, therefore, that molecular size does not affect appreciably the movement of these substances, after they have entered the conducting tissues. A case in point is the phloem-limited virus which moves readily through the sieve tubes, but must be placed there through the intervention of an insect vector. There are, of course, other viruses which are able to migrate in the parenchyma from cell to cell.

Thus it would seem that the selectivity of absorption and the subsequent transport are mediated to some extent by the intervening plant cells which must be traversed by the solute as it proceeds from the

absorbing surface to the phloem and xylem elements. The selectivity of these cells may help explain why Pramer,²⁸⁴ Crowdy,⁸⁰ and Dye¹¹¹ have observed the uptake of antibiotics through cut shoot surfaces to be greater than through intact plant root systems.

a. Upward Translocation

The antibiotics which have received the most attention with respect to translocation from roots and cut surfaces are streptomycin (S), griseofulvin (G), penicillin (P), chloramphenicol (CH), the tetracyclines (T), and cycloheximide (CY). Although the degree of transport has been shown to vary for a given antibiotic from one plant species to the next, certain similarities in the translocation pattern for all of the above antibiotics have been observed. These are listed below along with the antibiotic demonstrating the particular pattern.

1. The concentration detected in the aerial portion of the plant varied directly with the concentration of treating solution (S,G,P,CH,T, and CY).
2. Within certain limits, the concentration detected in aerial portions of the plant increased with time (S,G,P,CH,T, and CY).
3. Within certain limits, particularly early in the exposure period, a gradient of decreasing antibiotic concentration was established from base to apex (S,G,CH, and T).
4. Within certain limits, translocation appears to be a linear function of water uptake and the external factors influencing transpiration (G,CH).

Accumulation of antibiotics against a concentration gradient in the aerial portions of plants whose root systems have been exposed to these compounds has been definitely established by Dye¹¹¹ for the uptake of streptomycin by peach seedlings, and by Pramer²⁸⁴ for the uptake of the same antibiotic by cucumber seedlings. Similarly, Crowdy and Pramer⁸⁰ reported that tomato and lettuce plants exposed for 10–14 days in 25 mcg/ml streptomycin solutions contained 150 and 31 mcg/ml, respectively, of this antibiotic in the juices expressed from their foliage (see Table 4–9). Crowdy and co-workers⁸³ were able to obtain much higher concentrations of griseofulvin in the roots of bean plants than the concentration in the ambient solution. However, these high concentrations were attributed to the greater solubility of the antibiotic in lipoid media than in aqueous media. The low concentrations of griseofulvin detected in the cell sap tend to support this premise, as do additional experiments of Crowdy and Pramer,⁸⁰ who found griseofulvin concentrations in broad

TABLE 4-9

CONCENTRATION OF ANTIBIOTIC ASSAYED IN EXPRESSED LEAF SAP OF PLANTS TREATED FOR 10-14 DAYS WITH 25 MCG/ML

Plant	No. replicates	Mean concentration of antibiotic mcg/ml		
		Griseofulvin	Chloramphenicol	Streptomycin
Broad bean	5	0.3	20	0
Tomato	5	0.6*	11	150
Lettuce	5	0	5	31
Cabbage	5	0.5	4	5
Wheat	5	0.4*	5	19

* Mean of 3 replicates. (After Crowdy and Pramer⁸⁰.)

bean foliage from similarly treated plants to be 3,4, and 15 mcg/ml, respectively, in expressed juice, and in water and chloroform extracts. Thus, a simple diffusion mechanism could account for uptake of the compound. However, Crowdy *et al*⁸³ extended the disclosures of Stokes³²³ to show that the translocation of griseofulvin, measured by concentration in guttation water, is impeded by enzyme poisons such as 2,4-dinitrophenol and sodium azide. Using these respiratory inhibitors, it was possible to discern two separate processes involved in the uptake of griseofulvin by broad bean plants, as follows:

- a) An initial rapid entry into the roots, accomplished within 30 minutes, was inhibited by enzyme inhibitors.
- b) A prolonged uptake linearly related to transpiration, which was not affected by the inhibitory chemicals.

It is to be recalled that Pramer^{286,288} was able to elicit a similar effect upon streptomycin uptake by *Nitella* with catalytic amounts of 2,4-dinitrophenol and other respiratory poisons. Thus, it would appear that neutral organic molecules, as well as organic ions, are involved in an active transport situation.

b. Downward Translocation

There is little doubt that antibiotics applied to the roots or lower portions of the plant, which are eventually detected in the upper regions of the plant, reach their destination via the xylem. Furthermore, there seems to be little doubt that this movement is accomplished under the influence of the transpiration stream. Two questions which may be

asked concerning downward translocation of antibiotics are: (a) Does it occur?; and (b) What is the vascular route?

To test the possibility of downward translocation of antibiotics from leaf exposures, Mirzabekian and Menkova²⁵¹ placed antibiotic-saturated cotton pads on the upper leaves of tomato plants. Some of the treated plants were placed in humidity chambers, while others were not. After a 72-hour exposure, it was found that streptomycin had moved a distance of 8.5 cm down the stems of plants held in a saturated atmosphere. Conversely, plants maintained under conditions of lower relative humidity exhibited a slower and less extensive translocation. In similar exper-

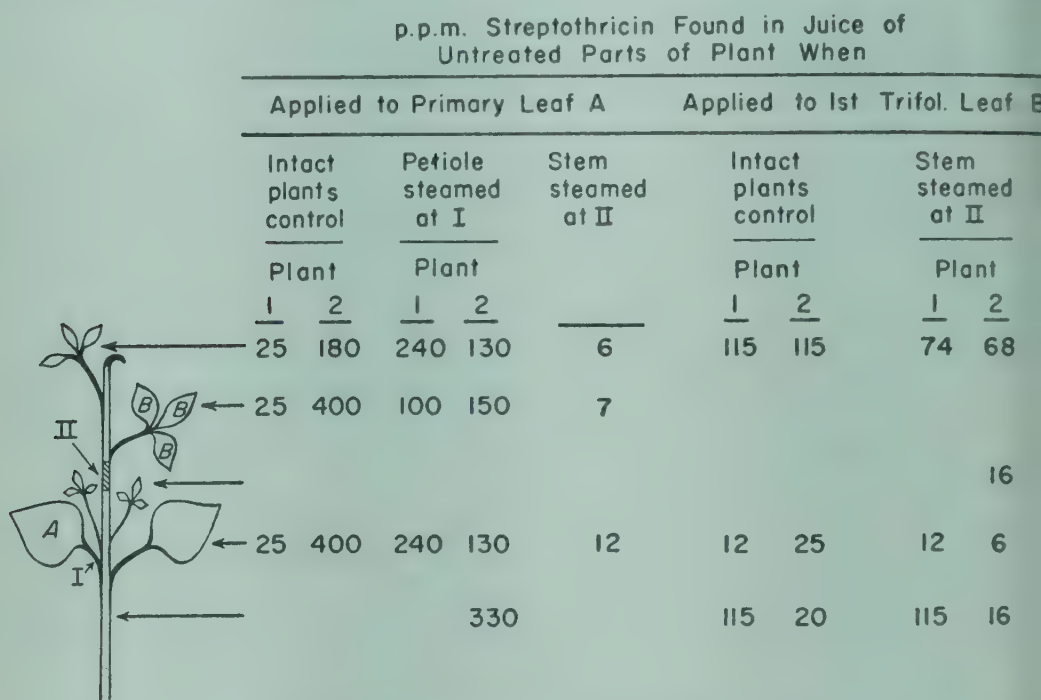


FIGURE 4-14. TRANSLOCATION OF STREPTOTHRICIN IN BEAN PLANTS.

ments, it was found that neither grizemin nor chlortetracycline move significantly from the treated leaves. The observation that streptomycin moved more readily from leaves of plants kept under high relative humidity than from those kept in a less saturated atmosphere suggests transport through the xylem. For if downward movement were to be accomplished through the xylem, it would occur more readily under conditions of reduced transpiration. Furthermore, downward movement in the xylem would be more likely to proceed if the roots were in a drier medium.

It has been reported by Gray¹⁶⁰ that both streptothricin and pleocidin were transported upwards and downwards in bean plants from primary

and trifoliolate leaves which had been immersed in tubes containing 1,000 and 2,000 mcg/ml solutions of these antibiotics.

In order to discern whether this movement was occurring in the xylem or phloem, sections of bean stem and petiole were killed with steam. In

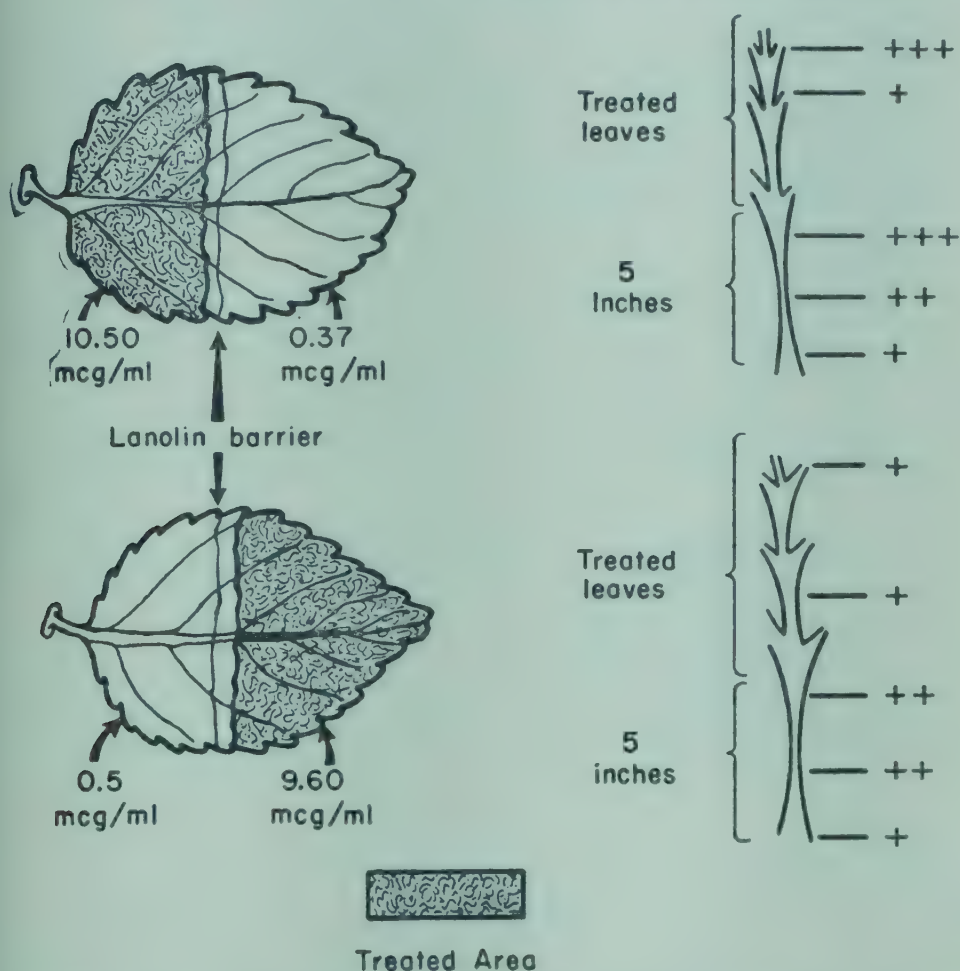


FIGURE 4-15. TRANSLLOCATION OF STREPTOMYCIN IN *Coleus* PLANTS. Lower surface application. A comparison of absorption and translocation by *Coleus* leaves from proximal and distal applications of streptomycin at 1,000 mcg/ml plus 1 percent glycerin to the lower surfaces. The antibiotic residues obtained from bioassay of expressed juice of 6 leaves* are indicated in mcg/ml for the area sampled. Drawings at the right indicate the relative amount of activity determined from 5 mm stem discs taken from designated areas. * Results represent an average of 2 experiments.

this way, the living sieve tubes were killed, assuring thereby that any appreciable transport would have to occur through the xylem. The fact that movement from immersed leaves did occur downwards through these killed sections certainly was indicative of xylem transport (see Fig. 4-14).

It is apparent that when leaf A is immersed in an antibiotic solution the movement of the transpiration stream to this leaf is reduced, and conceivably the antibiotic could be pulled by transpirational forces to the opposite primary leaf and upper trifoliate leaves. Further, if this

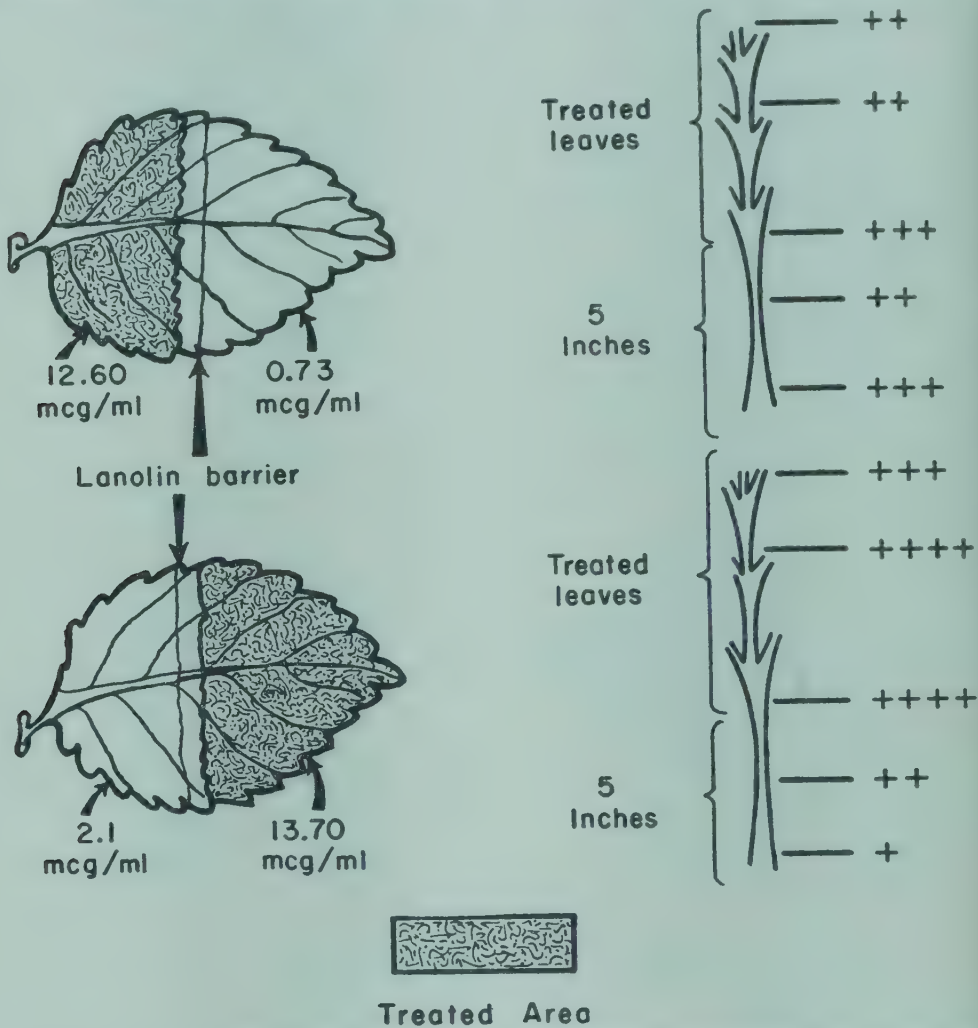


FIGURE 4-16. TRANSLOCATION OF STREPTOMYCIN IN *Coleus* PLANTS. Upper Surface Application. A comparison of absorption and translocation by *Coleus* leaves from proximal and distal applications of streptomycin at 1,000 mcg/ml plus 1 percent glycerin to the upper surfaces. The antibiotic residues obtained from bioassay of expressed juice of 6 leaves* are indicated in mcg/ml for the area sampled. Drawings at the right indicate the relative amount of activity determined from 5 mm stem discs taken from designated areas. * Results represent an average of 2 experiments.

movement is through the xylem, the killed sections at I and II need not impede passage of the antibiotic. Similarly, the immersed trifoliate leaf B may be giving up water to the trifoliate leaf above and to the primary leaf below under the influence of the pull exerted by these two

transpiring surfaces. It is difficult, however, to explain why transport is detected in the stem portions below the leaves and in the roots, if the root systems of these plants are amply supplied with water.

Dowler and Goodman^{101b} have recently detected downward translocation of streptomycin in *Coleus* (see Figs. 4-15 and 4-16). Stem disks showed antibiotic activity as far as 5 inches from the lowest treated leaf. The significant migration of streptomycin from distal leaf-halves to proximal portions is further evidence of downward translocation.

This movement was believed to occur in the phloem. It is hardly likely that movement in the quantity indicated could have occurred against the transpiration stream in 24 hours.

I. MODE OF ACTION

Mode of action as discussed in this chapter means the action exerted by the antibiotic in the plant as it suppresses the pathogen. This discussion is not concerned specifically with the mechanism of action against the microorganism *per se*, which is described in detail in another chapter.

Pramer, Robison and Starkey²⁸⁹ have enumerated the ways in which an antibiotic might inhibit a plant pathogen as follows:

1. By acting directly upon the pathogen.
2. By neutralizing toxins secreted by the pathogen.
3. By being transformed within the plant to a substance having greater or different activity.
4. By acting directly upon the host.

If one considers the experiments of Brown and Boyle⁵³ as the first studies of the application of antibiotics to plant disease control problems, there have been some 14 years during which research evidence has been accumulating on the subject of mode of action. The evidence that is now available may be examined conveniently in accordance with the "modes" listed above.

1. DIRECT ACTION OF THE ANTIBIOTIC UPON THE PATHOGEN

As early as 1944, Waksman and his associates³⁰⁸ reported plant pathogenic bacteria to be as sensitive to antibiotics *in vitro* as the bacteria which cause diseases of humans and animals. Since then a number of *in vitro* experiments have been conducted to evaluate the sensitivity of plant pathogens to antibiotics (see Table 4-10). Of these, the experiments of Katznelson and Sutton¹⁹⁷ were perhaps the most extensive. They evaluated six antibiotics against thirty-three species of phyto-

pathogenic bacteria. Their results indicated oxytetracycline and chlortetracycline to be extremely effective; however, streptomycin, neomycin and polymyxin were also highly effective, although their spectrum of activity was considerably more narrow than that of either of the tetracyclines. Species from each of the five genera of plant pathogenic bacteria were challenged by eight antibiotics in a study by Morgan and Goodman.²⁶⁶ The findings appear in Table 4-11, and

TABLE 4-10
SUMMARY OF *IN VITRO* EXPERIMENTS WITH ANTIBIOTICS

Antibiotic	Spectrum*	Investigations
Streptomycin	B. \pm	15, 17, 22, 97, 108, 110, 129, 135, 195, 196, 198, 206, 239, 256, 268, 307
Oxytetracycline	B. \pm	108, 110, 135, 195, 197, 206, 256, 307, 332, 339
Chlortetracycline	B. \pm	108, 110, 195, 197, 206, 234, 256, 307, 332
Chloramphenicol	B. \pm	108, 110, 195, 206, 234, 256, 332
Cycloheximide	F.	91, 148, 194, 313, 352, 353
Penicillin	B. \pm	52, 97, 195, 206, 234, 332
Polymyxin	B. -	108, 110, 195, 197, 206, 256
Clavacin (Patulin)	B. \pm , F.	129, 206, 300, 333, 343
Streptothricin	B. \pm	256, 298, 300, 343
Neomycin	B. -	195, 197, 256, 268, 307
Thiolutin	B. \pm , F.	108, 110, 135
Gliotoxin	B. \pm , F.	129, 300, 349
Filipin	F.	9, 10, 128, 152
Endomycin	B. \pm	91, 149, 220, 313
Dihydrostreptomycin	B. -	108, 110, 313
Candicidin	F.	224, 268, 313
Streptomyces sp.	B. \pm , F.	217, 293, 294
Erythromycin	B. \pm	91, 195
Tyrothricin	B. +	129, 206
Bacitracin	B. +	234, 268
Pelocidin	F.	268, 313
Fungichromin	F.	268, 313
Viomycin	B. \pm	256
Magnamycin	B. \pm	256
Tetracycline	B. +	195
Cathomycin	F.	268
Oxamycin	F.	268
Oligomycin	F.	268
Fradicin	F.	313
Ascosin	F.	313
Gramicidin	F.	313
Rimocidin	B. -, F.	342
Mycothricin	B. \pm	298
Antimycin	F.	215
Aspergillie acid	B. \pm , F.	129
Penicillie acid	B. \pm , F.	129
Toximycin	F.	321

* B., Bacteria; gram positive, (+); gram negative, (-); F., Fungi.

TABLE 4-11
SENSITIVITY OF BACTERIAL PLANT PATHOGENS TO ANTIBIOTICS

Test organism	Antibiotics							
	Aureo- mycin, meg/ml	Neomycin, meg/ml	Terra- mycin, meg/ml	Strepto- mycin, meg/ml	Polymyxin, meg/ml	Strepto- thricin, meg/ml	Viomycin, meg/ml	Chloro- mycetin, meg/ml
<i>Agrobacterium tumefaciens</i>	.025	.4	.05	.4	3.2	3.2	3.2	+12.5
<i>Corynebacterium michiganense</i>	.1	.1	.2	.05	.4	.4	.1	.8
<i>Erwinia amylovora</i>	.2	.25	.6	.2	.13	.1	1.2	.9
<i>Erwinia carotovora</i>	1.6	.4	6.3	.8	.2	.4	6.3	3.2
<i>Pseudomonas phaseolicola</i>	.1	.1	.2	.1	.1	.1	—	6.3
<i>Pseudomonas pisti</i>	1.6	.8	6.3	+12.5 ^a	.4	+12.5	1.6	+12.5
<i>Pseudomonas sesami</i>	.4	.2	.8	3.2	.4	.2	3.2	6.3
<i>Pseudomonas solanacearum</i>	.1	3.2	.8	.8	+12.5	.8	+12.5	+12.5
<i>Pseudomonas syringae</i>	.4	.1	.4	.2	.4	.8	1.6	6.3
<i>Xanthomonas campestris</i>	.2	.1	.8	.8	.2	1.6	12.5	6.3
<i>Xanthomonas malvacearum</i>	.05	.2	.8	.2	.2	.1	1.6	1.6
<i>Xanthomonas phaseoli</i>	.1	.1	.8	.1	.2	.2	.8	6.3
<i>Xanthomonas pruni</i>	.3	.5	1.4	.4	.51	.25	2.1	7.9
<i>Xanthomonas vicinicola</i>	.2	.1	.4	.4	.4	.2	+12.5	6.3

^a + 12.5 indicates inhibition not complete at that concentration. (After Morgan and Goodman²⁸⁸.)

suggest that the tetracyclines and streptomycin are particularly effective, inhibiting most species at less than 1 mcg/ml.

A direct effect of streptomycin upon the morphology of *E. amylovora* has been described by Goldberg and Morgan.¹³¹ With the aid of an electron microscope, these workers demonstrated that sub-inhibitory levels of the antibiotic induced profound morphological changes in the



FIGURE 4-17. ELECTRON MICROGRAPH SHOWING MORPHOLOGICAL EFFECTS OF STREPTOMYCIN ON *Erwinia amylovora*. Left: *E. amylovora* grown in 1 percent peptone at room temperature for 24 hours. ($\times 18,000$). Right: *E. amylovora* grown in 1 percent peptone and 0.025 mcg/ml streptomycin at room temperature for 24 hours. ($\times 13,200$).

bacterial cell. These changes were characterized by a series of lumps which developed in the cytoplasm, and which are clearly visible in Fig. 4-17.

Most of the existing *in vivo* data, particularly those concerning plant bacterial diseases, have been interpreted as resulting from the direct effect of the antibiotic on the pathogen. Disease control, accompanied by a concomitant inhibitory level of the antibiotic in the protected plant tissue, supports such a conclusion. However, one cannot dispute the view of Pramer, Robison and Starkey²⁸⁹ that this statement is only an assumption which has never been proved,

2. ACTIVITY DUE TO NEUTRALIZING TOXINS

In the literature reviewed by the author, no evidence was presented which suggested that antibiotic activity is exerted through detoxification. However, Brian and his group⁴⁹ offer the interesting speculation that antibiosis may result from the production of toxins. They offer in evidence the toxins with antibiotic properties, patulin (synonyms: clavacin, expansine), alternaric acid and fusaric acid. Their speculation is that the presence of these substances in plant tissue may discourage secondary invaders, preserving the host for the primary parasite.

3. TRANSFORMATION OF THE ANTIBIOTIC WITHIN THE PLANT

Although no data were found that proved unequivocally that some antibiotics are transformed within the plant, clear indications of this process exist. Gray¹⁶⁰ has recently reported that both the amine and oxime forms of streptomycin are converted in bean plants to materials with much higher antibiotic activity than that displayed by pure forms. These findings are based on bioassay data, and there is some reason to believe that the high activity ascribed to transformations of the amine and oxime reflects instead an unusually large accumulation of these compounds in the plant tissues assayed. More positive evidence of transformations is presented in the study conducted by Lemin and Magee²²⁵ with a labeled derivative of cycloheximide, cycloheximide acetate-2-C¹⁴. This compound was absorbed by tomato roots, and subsequently induced strong antifungal properties in the leaves. The conclusion that this activity was probably not due to the acetate form *per se* is supported by the following facts:

(a) Where low levels of the isotope were used, chromatograms detect cycloheximide 2-C¹⁴ only in the root extract.

(b) Bioassays reveal that cycloheximide (Rf 0.45) is active against *Saccharomyces pastorianus*, the test organism for the antibiotic, and that the acetate derivative (Rf 0.8) is inactive.

(c) Bioassay of leaf extracts revealed that the antifungal activity (Rf 0.45) showed a rapid increase with time (see Table 4-4).

The investigators conclude that cycloheximide acetate may be absorbed intact through the roots of the tomato plant, but that the antifungal activity detected is from free cycloheximide transformed within the plant.

A number of investigations have been reported in the literature in which *in vitro* experiments are successful and *in vivo* ones fail, suggesting

perhaps some modification of the antibiotic by the plant. The experience of Van Schaak³³⁵ demonstrates this situation; he found that both streptomycin and penicillin were effective *in vitro* against *Corynebacterium sepedonicum*. However, potato seed pieces inoculated artificially with this gram positive pathogen were protected by streptomycin and not by penicillin. The question arises whether or not the failure of penicillin was due to its inactivation within the plant tissue. One might assume that neutral penicillin, a specific compound for gram positive organisms, and which is shown below to be readily translocated, could have entered the tissues of the seed pieces through their exposed surfaces and then effected control.

Conversely, the antibiotic thiolutin was found to be more active *in vivo* by Gopalkrishnan and Jump.¹⁴⁷ The tomato wilt organism *F. oxysporium lycopersici* was inhibited at 10–20 mcg/ml *in vivo*, whereas similar inhibition was accomplished *in vitro* at 100 mcg/ml. A metabolite-antibiotic synergistic reaction is suggested, and the amino-acids histidine and methionine are proposed as the metabolites.

Finally, the experiments of Crowdy *et al.*,⁸² Pramer²⁸⁴ and Wallen and Millar,³⁴⁸ which were discussed in detail previously, strongly suggest that the chemical integrity of streptomycin, chloramphenicol, griseofulvin, and cycloheximide are maintained in plant tissue. Thus the activity observed in disease control and detected in bioassays can be ascribed to these compounds *au naturel*.

4. ACTION UPON THE HOST (INDIRECT ACTIVITY)

A considerable amount of data is beginning to accumulate indicating that some antibiotics act directly upon the host rather than the pathogen. In these instances it is the antibiotic-modified host which resists infection, or which limits the development of the pathogen. Information of this nature, as one might suspect, has been available for a number of years, but only in the light of recent developments have the real implications become clear.

The earliest *in vivo* experiments of Brown and Boyle^{53, 54} reported penicillin, produced in their own laboratory, inhibitory to gall growth induced by the crown-gall bacterium. Their *in vitro* tests established the gram negative *Agrobacterium tumefaciens* to be sensitive to penicillin. In a later series of experiments, Brown⁵⁶ used both penicillin and streptomycin, and showed that living crown gall cells, as well as the bacteria, were inhibited by the two antibiotics. Normal cells in or near the necrotic galls remained visibly uninjured. Thus, these antibiotics had apparently demonstrated a specificity for gall cells. Additional obser-

vations recorded at that time suggested that the antibiotics destroyed the nuclei of the gall cells. Using streptomycin, De Ropp⁹⁶ inhibited gall growth and described the action of this antibiotic as being generally inhibitory to the growth of embryonic tissue. In subsequent experiments⁹⁷ with streptomycin and penicillin, the former was found to be more inhibitory at 50 mcg/ml than the latter at 500 mcg/ml. From these experiments De Ropp concluded that the effect of streptomycin appears to be on the pathogen rather than on the host cells.

At this point it would seem that crown gall is inhibited by both a direct and an indirect action. Additional evidence of this nature is provided by an excellent series of experiments conducted by Klemmer, Riker and Allen.²⁰⁶ Their experiments showed that:

(a) Although more antibiotic was needed, oxytetracycline, chloramphenicol and polymyxin all inhibited completely the growth of bacteria-free galls grown in tissue culture.

(b) Growth of galls produced by chloramphenicol- and polymyxin-resistant strains of *A. tumefaciens* could be inhibited to at least 50% of normal by both chloramphenicol and polymyxin.

(c) Oxytetracycline, chlortetracycline, chloramphenicol, clavacin and streptomycin were more inhibitory to crown gall than polymyxin, penicillin and tyrothricin.

From the preceding experiments one might conclude that gall growth is suppressed by antibiotics through a direct action upon the bacterium and through an inhibitory effect upon the galls themselves. The extent to which either action proceeds seem to be governed by the specific antibiotic used, by the strain of *A. tumefaciens* inciting the gall or by whether or not the gall is a bacteria-free tissue culture.

1. *Stimulative and Other Effects:* It seems appropriate at this point to discuss briefly a few effects of antibiotics on higher plants that are not currently associated with disease control but which may be shown in the future to be quite intimately related to it.

Perhaps the best known of these phenomena is the stimulative effect that some antibiotics have on the growth of some plants, particularly at low concentrations. Stimulation of this type has been recorded under aseptic as well as non-sterile conditions, both with small aquatic plant forms and with agronomic species. The experiments of Nickell^{269, 270, 271} have been outstanding in this area and suggest that antibiotics have a direct effect on the metabolism of the plant, inducing thereby this growth stimulation. The possible mode of action and implications of this phenomenon will be discussed at length elsewhere in this book.

Havinga and co-workers,¹⁷⁰ using radiocarbon C¹⁴, noted that some antibiotics affect the dark CO₂ fixation process and the photosynthetic rate of the algae *Scenedesmus obliquies*. In these experiments penicillin did not alter the total uptake of CO₂, but changed the metabolic pattern in dark fixation; for example, a decrease in the formation of radioactive malic acid was observed. Chlortetracycline inhibited the dark fixation of CO₂ at concentrations of 10⁻³ M; however, it doubled the photosynthetic rate at 3×10⁻⁵ M; and the rate increased to six times (6×) that of the controls where a 1.5×10⁻⁴ M concentration was used. Another effect attributed to chlortetracycline was an abnormally large increase in sucrose production. In companion experiments oxytetracycline acted similarly.

TABLE 4-12

OXYGEN CONSUMPTION OF ASCORBIC ACID IN PRESENCE OF
6 ANTIBIOTICS (After Dudani and Krishnamurti¹⁰².)

Antibiotic added	Oxygen consumed, microliters	% activation + or inhibition -
Water (control)	25.0	—
Aureomycin hydrochloride	24.2	3.2
Chloramphenicol	25.7	+ 2.8
Neomycin sulphate	21.5	- 14.0
Dihydrostreptomycin sulphate	30.0	+ 20.0
Penicillin G	18.6	- 25.6
Terramycin hydrochloride	168.4	+ 573.6

The effect of antibiotics on the oxidation of ascorbic acid was evaluated by Dudani and Krishnamurti.¹⁰² Only oxytetracycline was found to catalyze the oxidation of ascorbic acid significantly. The order of effectiveness of the antibiotics studied appears in Table 4-12 and indicates that (a) the oxidation of ascorbic acid is an inherent property of the antibiotic itself; and (b) since this oxidative effect can be obtained with 1 mcg/ml, the reaction is catalytic in nature. In preservation studies conducted in the author's laboratory,¹³⁹ it was observed that oxytetracycline at 25 mcg/ml delayed the oxidative darkening of cabbage prepared as slaw and kept in sealed polyethylene containers.

Iyengar and Starkey¹⁹³ reported that antibiotics are able to synergize and antagonize the effects of auxin on *Avena* coleoptiles and pea seedlings. The synergistic effect was most evident with oxytetracycline and chloramphenicol, and was less pronounced with streptomycin. A reversal or inhibition of auxin (IAA) action was caused by the antibiotic, citrinin. The latter effect was more evident in the seedling pea test than the *Avena* section test.

2. *Effects of "Antibacterial" Antibiotics on Fungi:* With increasing frequency data are being presented that indicate a number of plant pathogenic fungi to be sensitive *in vivo* to antibacterial antibiotics.

Hillborn¹⁸⁵ has reported that *Rhizoctonia* infections of potato stems were reduced considerably by bacitracin, polymyxin and streptomycin. *Verticillium albo-atrum* was also suppressed by some of these essentially antibacterial compounds. Bonde³⁸ immersed cut shoots of potato in streptomycin and oxytetracycline for 6 and 72 hours. The 72-hour treatment with oxytetracycline protected the potato against artificial inoculations with *Phytophthora infestans*.

Müller and his group²⁵⁸ grew 35-day-old tomato and potato plants for 1 week in streptomycin solutions and challenged detached leaves from these plants with zoospores of *P. infestans*. The disease was inhibited in tissue assaying 4 mcg/ml of streptomycin, yet more than 200 mcg/ml of the antibiotic were required to inhibit spore germination, and 8 mcg/ml depressed hyphal growth only slightly. It was observed that infection is not inhibited by the presence of streptomycin in the tissue. Nevertheless, spread of the parasite was restricted after contact with host plasm, as compared with the diffuse spreading of the pathogen in untreated tissue. Significantly, it was found that young leaves responded more favorably than did older ones. The data suggest that the observed effects are due to:

(a) Lethal concentrations of streptomycin at cell surfaces rather than a uniform distribution throughout the cell (thus explaining rapid inactivation upon contact with host plasm), or

(b) A change induced by the streptomycin, which alters the host pathogen relationship, conferring thereby a degree of resistance to the host.

The latter proposition is strengthened by the report of Vörös and his associates,³⁴¹ who conducted a series of splendid experiments which demonstrated that streptomycin concomitantly decreased the rate of respiration and increased polyphenolase activity in potato leaf tissue and tuber discs (see Table 4-13 and 4-14). These data indicate that streptomycin exerts its protective effect via the polyphenol-polyphenolase system of the host plant. Presumably, the effect results in an increase in the concentration of fungicidal phenolic compounds, e.g., quinones, etc., in the tissues exposed to streptomycin.

Another line of reasoning is submitted by Zaumeyer and his associates,^{368,370,372} who found streptomycin effective *in vivo* against *Phytophthora phaseoli* (downy mildew of lima beans), as well as *P. infestans*. Their data indicated that streptomycin preparations of lower purity

TABLE 4-13
THE INFLUENCE OF STREPTOMYCIN IN POLYPHENOLASE ACTIVITY

Hours after treatment	Polyphenolase activity		Streptomycin content (mcg/g fresh wt.)
	Control	Treated	
24	0.81	1.02	Traces
72	0.90	1.61	40

Polyphenolase activity and streptomycin content in potato leaves treated with streptomycin. Enzyme activity is expressed as the increase in oxygen uptake upon addition of substrates (0.02% catechol and 0.6% hydroquinone) in cubic millimeters of oxygen per milligram (fresh weight) of tissue homogenate, per hour. (After Vörös *et al*³⁴¹.)

were more effective than the crystalline compound, indicating that the former may have contained an active principle that was lost in purification.

Another series of experiments delineating the fungitoxicity of streptomycin are those pertaining to the control of certain genera of the *Peronosporaceae*. The initial report in this area was by Grosso¹⁶³ to the effect that streptomycin protected tobacco plants against blue mold, *Peronospora tabacina*. This report was confirmed in the following year by Kirby.²⁰³ Shortly thereafter, Coe⁶³ showed that *Pseudoperonospora cubensis*, downy mildew of cucumber, was sensitive to streptomycin, and that copper had an additive effect (see Table 4-15). The sensitivity of *P. cubensis* was confirmed by Ark and Thompson,^{27,30} and Natti *et al*^{262, 263, 264, 265} found streptomycin to be the better of a number of antibacterial types as a protectant against *Peronospora parasitica* (downy mildew of

TABLE 4-14
THE INFLUENCE OF STREPTOMYCIN ON RESPIRATORY RATE AND POLYPHENOLASE ACTIVITY

Hours after treatment	Respiratory rate		Polyphenolase activity	
	Control	Treated	Control	Treated
3	68	65	28	30
24	64	40	32	102

Respiratory rate and polyphenolase activity in potato discs treated with streptomycin. Respiratory rate is expressed as cubic millimeters of oxygen per gram (fresh wt.) per hour. Enzyme activity is expressed as increase in oxygen uptake in cubic millimeters under identical conditions upon addition of substrates. (After Vörös *et al*³⁴¹.)

broccoli). Most recently, Horner and Maier¹⁸⁸ successfully eradicated *Pseudoperonospora humuli* (downy mildew of hops) with streptomycin.

3. *Possible Mechanisms Responsible for the Indirect Action of Streptomycin Against Fungi*: Vörös *et al* have presented evidence showing that streptomycin activity against *P. infestans* may be linked to an increase in polyphenolase activity. Their data suggest that the resulting increased concentration of oxidized phenolic compounds may account for the resistance in tomato and potato plants reported by Müller and his associates.²⁵⁸ They also suggest that the synergistic, or at least the additive, effect frequently encountered with streptomycin-copper combinations may be due to the fact that the phenolases are copper enzymes.

TABLE 4-15

ADDITIVE EFFECT OF STREPTOMYCIN-COPPER COMBINATIONS IN IMPROVING CONTROL OF DOWNY MILDEW OF CUCUMBER, *PSEUDOPERONOSPORA CUBENSIS* (After Coe⁶³.)

Treatment	Concentration	Disease control index
1. Agrimycin	100 ppm	3.63
2. Agrimycin	200 ppm	4.75
3. Tribasic copper sulfate	4# /100	6.00
4. Agrimycin plus tribasic copper	50 ppm: 2# /100	5.13
5. Agrimycin plus tribasic copper	100 ppm: 2# /100	6.38
6. Agrimycin plus tribasic copper	100 ppm: 2# /100	6.88
7. Check-no treatment	—	2.25
L.S.D. 5%		1.241
L.S.D. 1%		1.70

However, recent data presented by Bonde and Johnson,^{42a} concerning *in vivo* activity of streptomycin against *P. infestans*, indicate that this synergistic effect can be obtained with non-copper-containing fungicides, and that it is, therefore, not a response peculiar to copper.

It is also possible that the additive effect may be a reflection of copper-induced phytotoxicity, which is believed to be a denaturation of cellular protein. This increase in polyphenolase activity becomes an even more attractive hypothesis for induced resistance, if one considers that many plants exhibit an injury response characterized by a darkening of tissues. One might also recall at this point the profound streptomycin-induced injury of the embryonic and other rapidly-growing tissues that were discussed previously.^{171,192,295,306,329,340}

All attempts to control the Peronosporaceae with streptomycin have not been as successful as those just described. Cox⁷³ was unable to

protect lettuce against *Bremia lactucae*, (downy mildew) with streptomycin. However, a combination of this antibiotic and captan was effective where either chemical alone was not. This partial success notwithstanding, the activity of streptomycin against fungi *in vivo* seems to be more or less confined to a group of obligate parasites. Included in this group are two species of *Phytophthora*, *infestans* and *phaseoli*, which are incidentally in the same taxonomic order, the *Peronosporales*. *P. infestans* was regarded for many years as an obligate parasite, and *P. phaseoli* is classed by Yarwood³⁶² as one of the most difficult species of *Phytophthora* to grow in axenic culture.

It is possible that the inherent fastidiousness of these streptomycin sensitive fungi is their common link, and that the common link is in turn an extreme sensitivity to oxidized phenolic compounds? Or does the inhibitory effect of streptomycin on chlorophyll synthesis limit qualitatively or quantitatively a photosynthate fraction necessary for the growth and development of these pathogens?

J. ANTIVIRAL ACTIVITY OF ANTIBIOTICS

It has been established that certain of the large viruses infecting humans and animals are sensitive to the so-called broad spectrum antibiotics, e.g., the tetracyclines and chloramphenicol.

The efficacy of these antibiotics and of a number of others against some plant viruses has been somewhat less impressive. Nevertheless the results obtained to date suggest that the antiviral activity displayed by some antibiotics is not entirely without merit, and that additional investigations and screenings are in order.

According to the limited number of studies conducted thus far, it would appear that antibiotics suppress plant viruses directly by limiting their reproductive capacity, or indirectly by modifying the metabolism of the host in some way, and that their effects are not generally lasting ones.

Of nine antibiotics tested by Leben and Fulton²¹⁹ for inhibitory action against tobacco necrosis and tobacco ringspot viruses, only streptothricin and oxytetracycline prevented lesion production. Since oxytetracycline did not reduce the infectivity of either virus, and since streptothricin only altered the infectivity of the ring spot virus, the inhibitory effect of these antibiotics appears to be an indirect one.

It was suggested by the authors that virus multiplication and symptom development are dependent on plant respiration, and the inference was made that the two antibiotics interfere in some way with this process.

In additional experiments it was found that oxytetracycline inhibited multiplication of the tobacco mosaic virus (TMV). The results did not

however, preclude the possibility that oxytetracycline influenced the infection process rather than multiplication *per se*. Using leaf discs floated in antibiotic solutions, Schlegel and Rawlins³¹² observed that TMV was inhibited by MK-61, an antibiotic produced by an actinomycete, a *Nocardia* sp. Although adequate light doubled the rate of virus multiplication over that in the dark, light intensity did not modify the inhibitory effect MK-61 had on TMV multiplication. Therefore, it was concluded that, unlike some organic compounds, the inhibitory effect of this antibiotic might be on the virus rather than on the metabolism of the host. Kirkpatrick and Lindner²⁰⁵ introduced chloramphenicol by vacuum infiltration into cucumber plants that had been inoculated the previous day with a stone fruit virus (PLMV), and into tomato seedlings that had been inoculated with TMV. In both instances control of the virus was measured photometrically and biologically, and it was found that the titer of both viruses had been reduced approximately 40 percent by chloramphenicol at 10 mcg/ml.

Ken Knight¹⁹⁹ infected Lovell peach seedlings with the peach rosette virus by means of patch grafts and then dipped the seedlings into various antibiotic solutions for a period of 4 hours. The antibiotics evaluated were the three tetracyclines, streptomycin, endomycin and neomycin, as well as cycloheximide and Agrimycin. Those seedlings treated with the tetracyclines and Agrimycin made various degrees of recovery, ranging from temporary to complete and lasting. Streptomycin alone, neomycin, endomycin and cycloheximide were not effective; however, their failure may in some instances be attributed to the difficulty with which they are absorbed and translocated.

Noformycin, an antibiotic produced by a species of *Nocardia*, applied by Gray¹⁵⁷ as a foliar spray at 100 mcg/ml, was found to inhibit both the production of local lesions and systemic infections caused by southern bean mosaic virus and TMV. Sprays containing 125 mcg/ml of crystalline noformycin were equally effective. The effect of this antibiotic was similar to, but not as persistent as, thiouracil. Of particular significance was the fact that exposure of both viruses *in vitro* to 1,000 mcg/ml of the partially purified noformycin, or to 250 mcg/ml of the pure hydrochloride for 24 hours at 28°C or for 81 hours at 6°C, did not reduce infectivity of either virus. Evidence was also presented that the antibiotic was translocated from the base of a bean leaf to the tip, and in the reverse direction. Cytovirin, an antibiotic produced by an unidentified streptomycete, was evaluated by Gray¹⁵⁷ for its capacity to protect tomato from the spotted wilt virus and tobacco from TMV. Two sprays applied 2 hours after inoculation, and again 12 days later, suppressed symptom expression for as long as 7 weeks. A number of

unsuccessful attempts to inhibit plant viruses with antibiotics have also been reported.^{32,124,246}

K. THE DEVELOPMENT OF BACTERIAL RESISTANCE TO ANTIBIOTICS

The history of antibiotic therapy in humans and animals has regularly acknowledged and described the emergence of antibiotic-resistant bacteria during prolonged treatment, or from exposures to sub-inhibitory levels of these drugs.

Although the development of resistant forms of plant bacterial pathogens has been expected, definite proof of its occurrence in the field under natural conditions has yet to be demonstrated. Nevertheless,

TABLE 4-16
ANTIBIOTIC RESISTANCE OF *E. AMYLOVORA* AFTER 13
TRANSFERS (After English and Van Halsema¹¹⁵.)

Antibiotic environment	Inhibitory in mcg/ml	Concentration
	Isolate # 1	Isolate # 2
Streptomycin only	500	1000
Streptomycin and oxytetracycline 10%	3	10
Streptomycin and oxytetracycline 1%	10	15

since streptomycin is currently the antibiotic of choice in phytopathology for the control of bacterial diseases, and since this antibiotic is most likely to induce resistance, the threat of drug-resistance remains of particular concern.

The rate at which strains of bacteria have been known to develop resistance to streptomycin is astonishing. For example, 14 transfers of *E. coli* through ever increasing concentrations of this antibiotic finally led to the development of strains of this organism that could grow in broth containing 226,000 times as much streptomycin as was required to inhibit growth in the initial culture.^{289a} A similar *in vitro* experiment was conducted by English and Van Halsema¹¹⁵ with two isolates of *E. amylovora* and one of *X. vesicatoria*, which were sensitive to streptomycin *in vitro* at less than 1.0 mcg/ml.²⁴ They demonstrated that the addition of 1 or 10% oxytetracycline retarded the emergence of antibiotic resistance (see Table 4-16). Similarly, Abo El Dahab and Cox¹ incubated 2 isolates of *Pseudomonas solonacearum* for 24 hours at their minimal inhibitory concentrations of 12-25 and 6-12 mcg/ml of chloramphenicol. Prolonged incubation with this drug permitted the isolation of strains re-

sistant to the antibiotic. Doubling the concentration of chloramphenicol at each subsequent transfer permitted a step-wise increase of resistance to a subsequent transfer permitted a step-wise increase of resistance to a maximum tolerance of 500 mcg/ml. Of additional interest, pathogenic and non-pathogenic isolates were obtained from strains susceptible to chloramphenicol, strains moderately resistant to that substance, and strains highly resistant to it, suggesting that drug resistance and pathogenicity are unrelated. De Ropp⁹⁷ studied the sensitivity of 5 strains of *A. tumefaciens* to streptomycin, penicillin, chloramphenicol and chlor-tetracycline. These isolates demonstrated, in addition to variations in cultural characteristics, different degrees of virulence. Although large differences in sensitivity to the various antibacterial substances were observed, these differences were not correlated with virulence. In addition to a chloramphenicol-resistant strain of *A. tumefaciens*, Klemmer *et al*²⁰⁶ obtained by serial transfers a polymyxin-resistant variant. Measuring the activity of the two antibiotics by their suppression of growth of gall tissue, it was found that growth incited by sensitive strains was suppressed at lower concentrations than that induced by the resistant strains. As mentioned earlier, the inhibitive effect exhibited by these antibiotics against galls of resistant strains probably reflects an effect upon gall tissue *per se* rather than upon the pathogenic bacteria. The development of 2 streptomycin-resistant Xanthomonads, *X. citri* and *X. malvacearum*, has recently been reported by Rangaswami.²⁹⁹

Thus, it is quite apparent that plant bacterial pathogens are capable of developing resistance to antibiotics. It would seem to be of more than academic interest to follow up reports of the failure of streptomycin or other antibiotics to suppress, *in vivo*, pathogens that had previously responded favorably. One could conceivably detect resistant variants by systematically isolating pathogens from pockets of infection within large antibiotic-treated plots. Particular attention might well be paid, in this respect, to plantings where antibiotic therapy had been practiced for more than one growing season.

L. SPECIES INTERACTIONS DUE TO ANTIBIOTIC EXPOSURES

It would appear that mixed biological populations which are relatively enduring have attained a certain harmony or equilibrium. Occasional shifts in this equilibrium undoubtedly occur as a result of genetic or climatic fluctuations. Nevertheless, if the population survives these influences it once again establishes an equilibrium.

A close inspection of the function of any mixed population would reveal certain rigid spheres of dominance and areas of interdependence.

It is apparent, therefore, that the direct and specific suppression of one or more segments of a given population could accelerate and/or suppress the growth of still others.

The foregoing is applicable to mixed microbial populations. Thus, the suppression of one species provides additional substrate for another or reduces the growth of a symbiont.

Bonde and Malcolmson⁴² observed that streptomycin-treated potato seed pieces showed an increase in rot caused by a *Fusarium* sp., and *Phoma tuberosa*. One might assume that streptomycin had suppressed the bacteria which normally inhibit the growth of these fungi. To test this assumption, sterile potato discs were dipped into streptomycin, and control disks were placed in sterile water, and both were inoculated subsequently with *Fusarium* and *Phoma*. Under these aseptic conditions, the fungi still grew more luxuriantly on the streptomycin-treated disks, suggesting a direct stimulation. However, Hervey¹⁸⁰ has reported that where thiolutin was applied to soil at 25 mcg/ml, a total population count revealed 167 million microorganisms per gram, whereas the water controls yielded only 48 million. Nodulation of sweet clover plants was also improved by antibiotic exposure. Plants treated with bacitracin, thiolutin, and cycloheximide at 25 mcg/ml developed 9.0, 7.0, and 5.7 nodules per plant, respectively, as compared with 1.9 for the control series. It was suggested that these increases were due to the suppression of organisms which normally restrict the activity of microbes, such as *Azotobacter* and the nitrifying bacteria.

Goodman and Johnston¹⁴³ observed that whole potatoes dipped in cycloheximide underwent a more rapid soft-rot decomposition than those treated with streptomycin, or than the water-dipped controls. These results were interpreted as indicating an induced imbalance in the microflora, and/or a phytotoxic effect of the antibiotic. In additional experiments, Goodman *et al*¹⁴⁶ observed that, under similar conditions, potatoes treated with a cycloheximide-oxytetracycline combination remained in good condition for more than 4 months. On the other hand, those treated with cycloheximide decomposed in 2 months. These data indicate that the addition of oxytetracycline delayed the onset of soft-rot. It would appear, therefore, that the rapid decomposition observed in the cycloheximide-treated potatoes may be in part attributed to an induced imbalance in the indigenous microflora.

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65. Conover, Robert A., 1954, Control of bacterial spot of tomato and pepper seedlings with agrimycin. *Plant Disease Reporter* **38**, 405-409.

An account of field control of *X. vesicatoria* with Agrimycin.

66. Conover, Robert A. 1955, Control of bacterial spot in tomato fields with streptomycin-terramycin sprays. *Plant Disease Reporter* **39**, 611-615.

Although the per acre cost of the spray was \$74.40 the increase in yields and per cent disease-free fruit obtained more than offset the expense.

67. Couch H. B. and Cole, H., Jr., 1956, Five compounds tested for control of Merion bluegrass rust. *Plant Disease Reporter* **40**, 103-105.

Actidione applied twice a week at two week intervals at the rate of 422 or 844 mgm/2 gal/1,000 sq ft controlled rust of Merion blue grass.

68. Cox, R. S., Comegys, W. R. and Heuberger, J. W., 1953, Preliminary tests with antibiotics for the control of bacterial leaf spot of pepper. Transactions of Peninsula Horticultural Society, *Bul. Del. St. Bd. Agr.* **43**.

An early report on field control of *X. vesicatoria* with streptomycin.

69. Cox, R. S., 1955, Compatibility between a streptomycin-terramycin formulation and copper in the control of bacterial blight of celery. *Plant Disease Reporter* **39**, 484-486. Abstract.

A combination of copper and streptomycin gave superior control for bacterial blight of celery caused by *Pseudomonas apii*.

70. Cox, R. S., 1955, Compatibility between streptomycin and copper in the control of bacterial spot of pepper. *Plant Disease Reporter* **39**, 616.

Bi-weekly applications of streptomycin are less effective than weekly administrations and 200 mcg/ml appears to be the lowest effective dosage.

71. Cox, R. S., 1956, Progress in the control of bacterial spot of pepper in south Florida. *Plant Disease Reporter* **40**, 205-209.

A protective spray program with streptomycin is advocated for *X. vesicatoria* control. The effectiveness of the treatment can be markedly enhanced by adding a fixed copper to the spray formulation.

72. Cox, R. S., 1957, Additive effect of Agrimycin and copper in the control of bacterial spot of pepper. *Phytopathology* **47**, 6. Abstract.

The additive effect of Agrimycin and copper in controlling bacterial spot of pepper is recorded.

73. Cox, R. S., 1957, Control of downy mildew of lettuce in the Everglades. *Plant Disease Reporter* **41**, 445-459.

Neither streptomycin nor captan controlled lettuce mildew (*Bremia lactucae*); however, together these 2 compounds provided a significant degree of control.

74. Cox, R. S., Carroll, V. J. and Benedict, R. A., 1957, Studies on the etiology and control of the radish pit disease. *Phytopathology* **47**, 7. Abstract.

Oxytetracycline at 40 mcg/ml prevented the development of a bacterial pitting in radish. This treatment resulted in a residual level of the antibiotics in radish of 0.420 mcg/ml.

75. Cox, R. S. and Hayslip, N. C., 1957, Recent developments on the control of foliar diseases of tomato in South Florida. *Plant Disease Reporter* **41**, 878-883.

Streptomycin alone at 50-200 mcg/ml was ineffective in preventing *X. vesicatoria*. However, 50 mcg/ml plus 4 pounds/100 of tribasic copper was significantly more effective.

76. Crosier, Willard and Szkolnik, Michael, 1956, Sulfur, karathane and actidione for control of powdery mildew of wheat. *Plant Disease Reporter* **40**, 337-339.

Cycloheximide at 2 mcg/ml was inferior to Karathane and sulfur at 1 and 6 pounds/100 gal respectively for the control of powdery mildew of wheat.

77. Crossan, D. F. and Krupka, L. R., 1955, The use of streptomycin on pepper plants for the control of *Xanthomonas vesicatoria*. *Plant Disease Reporter* **39**, 480-483.

A paper disk assay of juices from pepper plants sprayed 3 times at 500 mcg/ml failed to disclose antibiotic activity in washed leaves. Lack of sensitivity of the test organism *X. vesicatoria* is suggested as a possible reason for this.

78. Crossan, D. F., Lloyd, P. J., Hyre, R. A. and Heuberger, J. W., 1957, Control of downy mildew of lima bean. *Plant Disease Reporter* **41**, 156-159.

Streptomycin at 100 mcg/ml plus 1% glycerin was not as effective as streptomycin plus 1½ pounds tribasic copper in reducing *Phytophthora phaseoli*.

79. Crosse, J. E., 1957, Streptomycin in the control of bacterial canker of cherry. *Annals Applied Biology* **45**, 226-228.

The leaf spot stage of *Pseudomonas mors-prunorum* seems more sensitive to streptomycin whereas Bordeaux is more effective in preventing the canker phase of the disease. Bordeaux is apparently more effective as a surface disinfectant.

80. Crowdy, S. H. and Pramer, D., 1955, The occurrence of translocated antibiotics in expressed plant sap. *Annals of Botany* **XIX**, 81-86.

A review article on translocation in which it is concluded that the neutral and acidic antibiotics are readily translocated whereas the basic and amphoteric ones have provided anomalous results.

81. Crowdy, S. H. and Pramer, D., 1955, Movement of antibiotics in higher plants. *Chemistry and Industry* **1955**, 160-162.

Assay of expressed sap for griseofulvin and chloramphenicol was found not to vary significantly from values obtained from water extracts of tissue. Water and organic solvent extracts of tissue for griseofulvin were found to vary greatly and a lipoid binding is postulated.

82. Crowdy, S. H., Gardner, D., Grove, John Frederick and Pramer, D., 1955, The translocation of antibiotics in higher plants. I. Isolation of

griseofulvin and chloramphenicol from plant tissue. *Jour. of Experimental Botany* **6**, 371-383.

Griseofulvin assayed spectrophotometrically and chromatographically provided results that were in good agreement with the preferred bioassay method.

83. Crowdy, S. H., Grove, John Frederick, Hemming, H. G. and Robinson, Kathleen, C., 1956, The translocation of antibiotics in higher plants. II. The movement of griseofulvin in broad bean and tomato. *Jour. of Experimental Botany* **7**, 42-64.

The amount of griseofulvin taken up by broad bean was proportional to the volume of water transpired. It was also found that the initial rapid entry of griseofulvin into roots could be inhibited by enzyme poisons such as sodium azide.

84. Crowdy, S. H. and Jones, D. Rudd, 1956, The translocation of sulphonamides in higher plants. I. Uptake and translocation in broad beans. *Jour. of Experimental Botany* **7**, 335-346.

Accumulation of sulfonamide in roots of bean plants appears to be related simply to time. Movement to stems and leaves depends upon transpiration.

85. Crowdy, S. H., 1957, The uptake and translocation of griseofulvin, streptomycin and chloramphenicol in plants. *Annals Applied Biology* **45**, 208-215.

At low concentrations, an appreciable lag in streptomycin absorption was observed. This was attributed to the need of saturating absorbing sites before free movement could proceed.

86. Daines, Robert H., 1956, Bacterial spot of peach (*Xanthomonas pruni*) and its control by the use of captan and streptomycin. *Plant Disease Reporter* **40**, 335-336.

Streptomycin at 150 meg/ml and 2 pounds of captan applied as a spray and captan at 4 pounds/100 gal were the best of a group of treatments for *X. pruni* control.

87. Daines, R. H. and Gray, R., 1957, Streptomycin foliage sprays and the control of bacterial spot of peach. *Phytopathology* **47**, 448.

The addition of glycerin improved the effectiveness of streptomycin in controlling *X. pruni*. Older leaves showed considerably more absorbed streptomycin than did young ones.

88. Davis, D. and Diamond, A. E., 1953, Inducing disease resistance with plant growth-regulators. *Phytopathology* **43**, 137-140.

Results with a series of growth regulators applied to tomato plants indicate that an induced alteration in metabolism of plant may also alter its disease susceptibility.

89. Davis, D. and Rothrock, W. J., 1956, Localized systemic activity of griseofulvin in the control of *Alternaria* blight of tomato. *Plant Disease Reporter* **40**, 328-331.

Griseofulvin applied to tomato plants at 1,000 meg/ml 24 hours prior to inoculation with spores of *Alternaria solani* provided near perfect disease control. At 125 meg/ml almost 90% control was afforded.

90. Davis, S. H., Engel, R. E. and Silber, G., 1951, Control of brown patch of turf in New Jersey. *Phytopathology* **41**, 657. Abstract.

Cycloheximide ranked 8th in a field of 9 fungicides evaluated for control of brown patch of bent glass caused by *Rhizoctonia solani*.

91. Davison, Arlen D. and Vaughn, John R., 1957, Effect of several antibiotics and other organic chemicals on isolates of fungi which cause bean root rot. *Plant Disease Reporter* **41**, 432-435.

Cycloheximide, ilotycin and endomycin were evaluated for activity against a *Fusarium* isolate with the latter being most effective.

92. de Beer, E. J. and Sherwood, M. R., 1945, The paper-disc agar-plate method for the assay of antibiotic substances. *Journal of Bacteriology* **50**, 459-467.

Factors which contribute to the precision of the paper-disc assay method are uniformity of volume absorbed by disc, constant depth of agar, uniform seed, and a linear-lag-dose response curve.

93. Deep, Ira W., 1958, Reduction in incidence of crown gall of Mazzard cherry following antibiotic treatments. *Plant Disease Reporter* **42**, 476-480.

Streptomycin sulfate, agrimycin and oxytetracycline were evaluated as control agents for crown gall of Mazzard cherry seedlings. All antibiotics significantly reduced infection; however, oxytetracycline was superior.

94. Dekker, J., 1955, Internal seed disinfection by an antibiotic from *Streptomyces rimosus*. *Nature* **175**, 689-690.

Seed soaks of 18 hours with purified rimocidin and a substance produced by *S. rimosus* both reduced the incidence of *Ascochyta pisi* and *Mycosphaerella pinodes* on pea seeds.

95. Dekker, J., 1957, Internal seed disinfection of peas infected by *A. pisi* by means of the antibiotics rimocidin and pimaricin, and some aspects of the parasitism of this fungus. *Tijdschr. Plantenziekten* **63**, 65-144.

Polyene antibiotics rimocidin and pimaricin applied at 75 mcg/ml for 24 hours reduced the incidence of *A. pisi* from 15-40% to 0.5-1.5%. The fungus was completely eradicated by soaking at 35°C for 48 hours, but germination was impaired.

96. de Ropp, R. S., 1948, Action of streptomycin on plant tumors. *Nature* **162**, 459-460.

Data suggested that streptomycin is a general inhibitor of the growth of embryonic plant tissue rather than a specific inhibitor of tumor tissue.

97. de Ropp, R. S., 1949, The action of antibacterial substances on the growth of *Phytoplasma tumefaciens* and of crown gall tumor tissues. *Phytopathology* **39**, 822-828.

Streptomycin has only a short eradivative potential of 2 days as demonstrated by tissue culture data. The effect of streptomycin appears to be upon the pathogen rather than on the host cells.

98. de Zeeuw, Donald J. and Vaughn, John R., 1950, An antibiotic of potential value in a field control of cucumber scab. *Plant Disease Reporter* **34**, 7-8.

Cycloheximide applied at 10 mcg/ml significantly reduced the incidence of *Cladosporium cucumerinum*, the causal organism for cucumber scab.

99. Di Marco, G. R. and Davis, B. H., 1957, Prevention of decay of strawberries with post-harvest treatments. *Plant Disease Reporter* **41**, 460-464.
Mycostatin at 100 mcg/ml applied post-harvest to strawberries improved the storage condition of the fruit by delaying growth of *Botrytis* and *Rhizopus* molds.
100. Doolittle, S. P. and Beecher, F. S., 1955, Effect of streptomycin formulations on angular leaf spot in cucumber. *Plant Disease Reporter* **39**, 731-736.
Foliar sprays with 400 mcg/ml of streptomycin significantly reduced infection from artificial inoculations with *Pseudomonas lachrymans*. An eradivative potential was observed when 2 sprays at 400 mcg/ml were applied.
101. Dosdall, Louise D., 1955, Calla rhizome treatments. *Plant Disease Reporter* **39**, 779-780.
A one-hour dip of calla rhizomes in 5 mcg/ml of cycloheximide was extremely phytotoxic.
- 101a. Dowler, W. M., 1958, Thesis. Studies on the absorption, translocation and detection of antibiotics in plants. University of Missouri. (Unpublished.)
- 101b. Dowler, W. M. and Goodman, R. N., 1958. Downward translocation of streptomycin in *Coleus* sp. *Phytopathology* (in press).
A study of the movement of streptomycin comparing upper and lower leaf surface applications to *Coleus* sp. was conducted. The antibiotic applied to either proximal or distal portions of leaves was detected after 24 hours in the untreated portion and in stem discs taken approximately 5 in. below the treated leaves.
102. Dudani, A. T. and Krishnamurti, C. R., 1954, Oxidation of ascorbic acid by terramycin. *Biochimica et Biophysica Acta* **13**, 505-509.
Of 6 crystalline antibiotics tested only oxytetracycline catalyzed the oxidation of ascorbic acid. A number of enzyme poisons failed to inhibit this oxidation. The catalytic activity is thermostable.
103. Dunegan, John C. and Wilson, R. A., 1953, Some effects resulting from the introduction of antibiotics into fruit trees. *Phytopathology* **43**, 405. Abstract.
An Elberta peach tree absorbed 1.7 grams of oxytetracycline in 28.4 liters of water which caused the foliage to show a yellow-green mottle and a reduction in defoliation due to *X. pruni*.
104. Dunegan, John C., Wilson, R. A. and Morris, W. T., 1953, Effects of terramycin on peach trees affected with bacterial spot. *Plant Disease Reporter* **37**, 604-605.
Oxytetracycline solutions were not as readily absorbed through holes in tree trunks of peach trees as the plain water controls. Only 7-10 liters of the antibiotic solution were absorbed whereas the controls absorbed 40 liters of water.
105. Dunegan, John C., 1954, Antibiotics in plant disease control. *Jour. of Agr. and Food Chem.* **2**, 1020-1022.
A general review article on the role of antibiotics in plant pathology.
106. Dunegan, John C., Kienholz, Jess R., Wilson, R. A. and Morris, W. T., 1954, Control of pear blight by a streptomycin-terramycin mixture. *Plant Disease Reporter* **38**, 666-669.
Over the same period of time, 3 sprays at 2 week intervals at 100

mcg/ml were as effective as 5 sprays at weekly intervals at 30 mcg/ml in the control of pear blight, caused by *E. amylovora*.

107. Dunegan, John C. and Wilson, R. A., 1956. Preliminary note on the downward movement of streptomycin in apple and pear tissue. *Plant Disease Reporter* **40**, 478.

A thread wick infused streptomycin from a vial into a puncture at the base of a leaf blade and induced chlorosis in that leaf and in leaves 4-8 nodes above and 2-4 nodes below. Intensity of chlorosis varied with the concentration.

108. Dye, D. W. and Dye, M. H., 1954, Effectiveness of therapeutants including antibiotics in preventing development of blast of stone fruit (*Pseudomonas syringae* van Hall). *New Zealand Jour. of Sci. and Tech.* **36**, 21-26.

Of 6 antibiotics tested streptomycin and dihydrostreptomycin were most effective in preventing the development of "blast" of peaches caused by artificial inoculations with *Pseudomonas syringae*.

109. Dye, D. W., 1956, Blast of pear. *The Orchardist of New Zealand*, August, 1956.

P. syringae which causes pear blast was significantly reduced by streptomycin at 100 mcg/ml in a 3-spray schedule with applications made at open cluster, full bloom, and petal fall.

110. Dye, M. H., 1954, *In vitro* studies of the effect of antibiotics on *Pseudomonas syringae*. *New Zealand Jour. of Sci. and Tech.* **36**, 27-31.

Streptomycin serially diluted was found to be bactericidal to an inoculum of 2.05×10^6 cells of *P. syringae* at a concentration of 5 mcg/ml after 48 hours.

111. Dye, M. H., 1956, Studies on the uptake and translocation of streptomycin by peach seedlings. *Annals of Applied Biology* **44**, 567-575.

Adding macerated leaf tissue to streptomycin solutions decreased the amount of streptomycin detectable in the supernatant liquid. Adsorption of the antibiotic to the tissue is suggested as a reason for this decrease.

112. Dye, M. H., 1956, Recent work with horticultural formulations of streptomycin. *The Orchardist of New Zealand*, Dec., 1956.

Data suggest that streptomycin absorbed by the plant is not leached out by precipitation nor is it destroyed by the actinic rays of the sun.

113. Dye, M. H., 1956, Intake of streptomycin by peach leaves. *Nature* **178**, 551-552.

Whole peach leaves immersed in streptomycin solutions did not reduce the antibiotic concentration whereas macerated tissue did. This reduction was not entirely due to dilution or pH change.

114. Dye, M. H., 1957, Studies on horticultural formulations of streptomycin. *New Zealand Jour. of Sci. and Tech. Sec. A* **38**, 898-907.

Three formulations and a crude preparation of streptomycin sulfate were effective in preventing infection of peach leaves by *P. syringae* when applied as sprays at 100 and 50 mcg/ml before inoculation.

115. English, A. R. and Van Halsema, G., 1954, A note on the delay in the emergence of resistant *Xanthomonas* and *Erwinia* strains by the use of streptomycin plus terramycin combinations. *Plant Disease Reporter* **38**, 429-431.

Using 2 strains of *E. amylovora* and one of *X. vesicatoria*, it was shown that the addition of 1 or 10% oxytetracycline to streptomycin significantly retarded the emergence of antibiotic resistance.

116. Epps, William M., 1957, Control of potato seed piece decay in South Carolina 1952-1956. *Plant Disease Reporter* **41**, 148-150.

Streptomycin dips of an instant, 1 and 5 minutes and dusts were effective at 50 and 100 mcg/ml in improving stand and yield where conditions prevail for *E. atroseptica* and *E. caratovora* to flourish.

117. Epstein, Emanuel and Hagen, C. E., 1952, A kinetic study of the absorption of alkali cations by barley roots. *Plant Physiology* **27**, 457-474.

A hypothesis is presented for an absorption process mediated by an ion binding carrier system. The data suggest several distinct binding sites which exhibit preference, for example, of K, Rb, and Cs to Na and Li.

118. Esau, Katherine, 1953, Plant Anatomy. John Wiley and Sons, Inc., New York, New York. 735 pp.

A plant anatomy text book.

119. Esau, K., Currier, H. G. and Cheadle, V. I., 1957, Physiology of phloem. *Annual Review of Plant Phys.* **8**, 349-374.

The five major theories on phloem transport are presented. The morphology of the phloem components is discussed as well as the movement of such organic molecules as dyes, viruses, and radioactive substances.

120. Felber, I. M. and Hamner, C. L., 1948-49, Control of mildew on bean plants by means of an antibiotic. *Botanical Gazette* **110**, 324-325.

Apparently the earliest report of cycloheximide activity *in vivo* against a plant pathogen. A concentration of 10 mcg/ml of this antibiotic applied to bean leaves completely eradicated a mildew infection caused by *Erysiphe polygoni*.

121. Ferus, Charles L., Cole, Herbert, Jr. and Stambaugh, William J., 1955, The influence of actidione and other chemicals upon the oak wilt fungus. *Plant Disease Reporter* **39**, 491-494.

Cycloheximide completely inhibited germination of conidia and ascospores of oak wilt caused by *Ceratocystis fagacearum* at 1.0 mcg/ml. Previously established growth *in vitro* was inhibited 76% at a concentration of 0.01 mcg/ml and mycelial invasion of sapwood was inhibited 90% by 10 mcg/ml.

- 121a. Ford, Jared H., Klomparens, William and Hamner, Charles L., 1958, Cycloheximide (acti-dione) and its agricultural uses. *Plant Disease Reporter* **42**, 680-695.

An extensive review of most of the research accomplished to date with cycloheximide.

122. Frey-Wyssling, A., 1948, Submicroscopic Morphology of Protoplasm and Its Derivatives. Elsevier Publishing Company, Inc., New York, New York. 255 pp.

A monograph on the fundamentals of submicroscopic morphology. With the aid of the electron microscope, polarizing microscope and x-ray camera, information regarding the fine structure of protoplasm and protoplasmic derivatives is presented.

123. Fulton, Robert H., 1951, Comparison of fungicides for control of powdery mildew on the Latham red raspberry in 1950. *Plant Disease Reporter* **35**, 538-539.

Powdery mildew of raspberry caused by *Sphaerotheca humuli* was insensitive to cycloheximide at 5 mcg/ml.

124. Fulton, R. H., 1954, Studies on inactivation of the strawberry type 2 virus *in vivo*. *Phytopathology* **44**, 489. Abstract.

The strawberry type 2 virus was insensitive to cycloheximide, chlortetracycline, neomycin and streptomycin at 250 mcg/ml.

125. Fukunaga, D., Misato, T., Ishili, I. U. and Asakawa, M., 1957, Application of antibiotics as agricultural chemicals. On the studies of blasticidin, a new antiphytopathogenic fungal substance. IV International Congress of Crop Protection, Hamburg, Germany. Summary of paper 2, pp. 212.

Blasticidin A is a selection from 140 actinomycetes which produce antifungal substances and which were originally screened from 7,243 actinomycete strains at Tokyo University. It appears to be active against rice blast which is caused by *Piricularia oryzae*.

126. Garber, J. D., Rothrock, J. W., Reynolds, H. C. and Gray, R.A., 1954, Laboratory and field studies of agricultural streptomycin. *Agr. Chemicals* **9**, 32-34.

The efficacy of various streptomycin formulations with respect to their solubility and concomitant release of the compound for plant absorption is discussed. In addition to water soluble forms some oil-soluble fatty acid salts of streptomycin are described.

127. Gasiorkiewicz, E. C., 1956, Effects of antibiotic dip treatments on carnations. *Plant Disease Reporter* **40**, 421-423.

Streptomycin inhibited root formation on some varieties of carnation cuttings at concentrations as low as 1 mcg/ml. Complete inhibition of root formation was observed at 20 mcg/ml.

128. Gattani, M. L., 1957, Studies on the control of damping-off of safflower with antibiotics. *Plant Disease Reporter* **41**, 160-164. Abstract.

Periodic irrigation of soil with filipin at 50 mcg/ml effectively controlled the pre-emergence stage of *Pythium* damping off. A three-week retardation of the fungus was afforded by this treatment.

129. Gilliver, K., 1946, The inhibitory action of antibiotics on plant pathogenic bacteria and fungi. *Annals of Botany* **10**, 271-282.

The inhibitory powers of 13 antibiotic substances on 33 causal organisms of plant disease are listed. The serial dilution method was used and bacteria were inhibited by more substances at higher dilutions than fungi.

130. Gilmer, R. M., 1955, Evaluation of various fungicides and application schedules in the control of diseases of cherry nursery stocks. *Plant Disease Reporter* **39**, 762-770.

A combination of 2 mcg/ml cycloheximide and 2-5 pounds of sulfur per 100 gal applied 4 times at 2 week intervals gave excellent control of cherry leaf spot and mildew of cherry.

131. Goldberg, Herbert S. and Morgan, Billie S., 1954, Change in bacterial morphology as a result of low concentrations of streptomycin. *Journal of Bacteriology* **68**, 507-508.

Sublethal or noninhibitory concentrations of streptomycin caused marked changes in the morphology of cells of 18–24 hour cultures of *Erwinia amylovora*. Electron micrographs showed a series of “lumps” in the cytoplasm of treated cells.

132. Goodman, J. J. and Henry, A. W., 1947, Action of subtilin in reducing infection by a seed-borne pathogen. *Science* **105**, 320.

Subtilin applied to barley seed artificially infested with *Xanthomonas translucens* significantly reduced the incidence of disease when used at a concentration of 1 : 1,000.

133. Goodman, Robert N., 1953, Antibiotics, a new weapon for fire blight control. *Am. Fruit Grower* **73**, 7, 16, 17.

Streptomycin at 100 mcg/ml applied 3–4 times during the apple blossom period gave near perfect control of fireblight caused by *E. amylovora*.

134. Goodman, Robert N., 1954, Fireblight control with sprays of agrimycin, a streptomycin-terramycin combination. *Plant Disease Reporter* **38**, 874–878.

Very little difference in the degree of fireblight control could be discerned between 4 and 3 spray schedules with streptomycin at either 100 or 50 mcg/ml. The eradivative potential of streptomycin for *E. amylovora* is negligible.

135. Goodman, Robert N., 1954, Antibiotics for control of fireblight. *Proc. Am. Soc. Hort. Sci.* **64**, 186–190.

Foliar sprays with streptomycin and oxytetracycline were capable of controlling fireblight in artificially inoculated one-year-old apple trees. Methyl cellosolve at 1% appeared to increase the effectiveness of the spray formulation.

136. Goodman, Robert N., 1954, Development of methods for use of antibiotics to control fireblight. University of Mo. College of Agr. *Research Bulletin* 540.

Greenhouse experiments established the fact that streptomycin and oxytetracycline acted as “systemic protectants” providing a period of immunity of at least 24 hours duration.

137. Goodman, Robert N. and Hemphill, D. D., 1954, The effects of indole-3-acetic acid on the plant disease-inhibiting properties of antibiotics. *Science* **119**, 347–348.

The data suggested that indole-3-acetic acid plays a role in the disease-inhibiting action of antibiotics and possibly the disease-inhibiting mechanism of the plant.

138. Goodman, Robert N., 1955, An effective spray schedule for antibiotic application to control fireblight of apple. *Ind. Microb. Soc. Proc.* **1955**, 39. Abstract.

On the basis of three years' experience in field control of fireblight a 4-spray schedule was formulated as follows: streptomycin at 50 mcg/ml is applied at full pink stage with successive sprays at 50, 25, and 25 mcg/ml at 5, 5, and 7 day intervals.

139. Goodman, Robert N., Unpublished data from experiments conducted in the laboratory, greenhouse, and field during the period 1953–1958.

140. Goodman, Robert N., 1955, Late season twig-infection, a serious limitation to the effectiveness of antibiotic sprays for fireblight control. *Plant Disease Reporter* **39**, 922-925.

Late season twig infection caused by *E. amylovora* may be independent of blossom blight and streptomycin will reduce only the blossom infection phase of this disease.

141. Goodman, Robert N. and Shepard, P., 1956, Indications of *Xanthomonas pruni* control with antibiotic sprays. *Plant Disease Reporter* **40**, 93-102.

Initial evidence is presented suggesting that the severity of bacterial spot of peach caused by *X. pruni* may be reduced by streptomycin sprays at 50 or 100 mcg/ml. The spray schedule was initiated 30 days after petal fall with successive sprays applied following significant rainfall.

142. Goodman, Robert N., 1956, Effects of organic fungicides and antifungal antibiotics on mushroom mildew *Dactylium dendroides*, and lipstick mold, *Geotrichum* sp. *Plant Disease Reporter* **40**, 714-717.

A number of antifungal antibiotics were applied as bed drenches for the control of mushroom mildew caused by *Dactylium dendroides*. None were found particularly effective nor was phytotoxicity discerned.

143. Goodman, R. N. and Johnston, M. R., 1956-57, Stability of streptomycin in apple and potato tissue. *Antibiotics Annual 1956-57*. Medical Encyclopedia, Inc., New York, 1006-1009.

A total of 0.375 mg of streptomycin sulfate was administered to 10-year-old Jonathan apple trees via the capsules in bore-holes in their trunks. Antibiotic activity was detected in fruit and foliage 4 months later.

144. Goodman, R. N. and Dowler, W. M., 1957, The influence of plant growth relating substances and hydroxy-compounds on the absorption of streptomycin by plants. IV. International Congress of Crop Protection, Hamburg, Germany. Summary of paper 6, p. 213.

It is suggested that hydroxy compounds improve streptomycin absorption by plants by increasing the degree of hydration of the waxy cuticle and the lipid fraction of the cell wall.

145. Goodman, R. N. and Dowler, W. M., 1958, The absorption of streptomycin by bean plants as influenced by growth regulators and humectants. *Plant Disease Reporter* **42**, 122.

Two growth regulators indole-3-acetic acid and gibberellic acid increased the absorption of streptomycin applied as a spray to bean plants by more than 2½ times.

146. Goodman, R. N., Johnston, M. R. and Goldberg, H. S., 1957-58, Residual quantities of antibiotics detected in treated plant tissue. *Antibiotics Annual 1957-58*. Medical Encyclopedia, Inc., New York, 236-240.

Spinach dipped in a streptomycin solution of 1,000 mcg/ml for 1 minute and for 15 seconds, then blanched in live steam for 3 minutes and rinsed in cold water showed residual antibiotic activity of 40 and 25 mcg/ml respectively.

147. Gopalkrishnan, K. S. and Jump, J. A., 1952, The antibiotic activity of thiolutin in the chemotherapy of the *Fusarium* wilt of tomato. *Phytopathology* **42**, 338-340.

Thiolutin appears to be more active *in vivo* than *in vitro* against *Fusarium* wilt of tomato. A metabolite-antibiotic synergistic reaction is suggested and the amino acids histidine and methionine are proposed as the metabolites.

148. Gottlieb, D., Hassen, H. and Linn, M. B., 1950, Actidione as a plant protectant. *Phytopathology* **40**, 218-219.

Six plant pathogenic fungi were evaluated *in vitro* for their sensitivity to cycloheximide. In addition, the sensitivity of tomato, bean, geranium, peach and strawberry to this antibiotic was observed. Strawberry showed no phytotoxic effects at 1000 mcg/ml.

149. Gottlieb, D., Bhattacharyya, P. K., Carter, H. E. and Anderson, H. W., 1951, Endomycin a new antibiotic. *Phytopathology* **41**, 393-400.

Endomycin isolated from a *Streptomyces* species inhibits the growth of a wide variety of fungi pathogenic to plants and animals and to many gram positive and a few gram negative bacteria.

150. Gottlieb, D., 1952, The disappearance of antibiotics from soil. *Phytopathology* **42**, 9. Abstract.

Basic antibiotics such as streptomycin and streptothricin are inactivated by the clay, the organic matter, and the microflora of the soil.

151. Gottlieb, David, Siminoff, Paul and Martin, Mary M., 1952, The production and role of antibiotics in soil. IV. Actidione and clavacin. *Phytopathology* **42**, 493-496.

Neither cycloheximide nor clavacin was removed from aqueous solution by the addition of soil. These antibiotics were not adsorbed by either montmorillonite or illite clays.

152. Gottlieb, D., Ammann, Alfred and Carter, H. E., 1955, A new antifungal agent, filipin. *Plant Disease Reporter* **39**, 219.

The initial publication noting the discovery of filipin, an antifungal antibiotic which has many properties that favor its use as a disease control agent of plants.

153. Gray, Reed A., 1955, Inhibition of root growth by streptomycin and reversal of the inhibition of manganese. *American Journal of Botany* **42**, 327-331.

Inhibition of root growth caused by as little as 20 mcg/ml of streptomycin could be overcome by the addition of 5 times as many moles of manganese. The calcium ion⁺⁺ was also partially effective in this respect. A number of other ions and organic metabolites were found to be ineffective.

154. Gray, Reed A., 1955, Increasing the effectiveness of streptomycin against the common blight of beans with glycerin. *Plant Disease Reporter* **39**, 567-569.

The addition of glycerin to streptomycin sprays increased the effectiveness of the antibiotic against *Xanthomonas phaseoli*, the causal organism of common blight of beans.

155. Gray, R. A., 1955, The downward translocation of antibiotics in plants. *Plant Physiology* **30**, vi. Abstract.

Streptothricin and pleocidin applied as sprays to tobacco, tomato and bean foliage were translocated both up and down the stem. Under similar conditions streptomycin, dihydro-streptomycin, neomycin, and other antibiotics were not translocated.

156. Gray, R. A., 1955, Increasing the absorption of streptomycin by leaves and flowers with glycerol. *Ind. Microbiol. Soc. Proc.* **41**. Abstract processed.

A significant increase in streptomycin absorption apparent 3 hours after bean leaves were sprayed with streptomycin plus glycerin. In 6 hours a 5-fold increase in absorption was detected where glycerin was added to a 500 mcg/ml spray.

157. Gray, Reed A., 1955, Activity of an antiviral agent from *Nocardia* on two viruses in intact plants. *Phytopathology* **45**, 281-285.

Tobacco mosaic virus and southern bean mosaic virus were inhibited by noformycin when applied 1 hour or 1 day after inoculation or if sprays were applied several days before inoculation.

158. Gray, Reed A., 1956, Increasing the absorption of streptomycin by leaves and flowers with glycerol. *Phytopathology* **46**, 105-111.

In addition to glycerol, hydroxy-compounds such as sorbitol, diethylene glycol and other polyhydroxy alcohols were effective in increasing the absorption of streptomycin by bean leaves. Wetting agents were usually without effect in this respect.

159. Gray, Reed A., 1957, Combating plant virus diseases with a new antiviral agent, Cytovirin. *Plant Disease Reporter* **41**, 576-578.

Southern bean mosaic virus was inhibited in the local lesion test as much as 90% by 0.5 mcg/ml of cytovirin. Tobacco mosaic virus lesions were inhibited 95% by 0.12 mcg/ml of this antibiotic.

160. Gray, Reed A., 1958, The downward translocation of antibiotics in plants. *Phytopathology* **48**, 71-78.

When primary leaves of bean plants were immersed in vials containing 1,000 mcg/ml of either streptothricin or pleocidin the antibiotics were translocated downward to the roots and upward to younger leaves. Xylem transport was suggested.

161. Gregory, K. F., Allen, O. N., Riker, A. J. and Peterson, W. H., 1952, Antibiotics as agents for the control of certain damping-off fungi. *American Journal of Botany* **39**, 405-415.

Stability in soil of cycloheximide and fradecin is not greatly influenced by soil type or pH.

162. Gregory, K. F., Allen, O. N., Riker, A. J. and Peterson, W. H., 1952, Antibiotics and antagonistic microorganisms as control agents against damping-off of alfalfa. *Phytopathology* **42**, 613-622.

Concentrations of cycloheximide of 6.2 and 25.0 mcg/ml in soil solution completely controlled damping-off of alfalfa caused by *Pythium debaryanum*. However, seedlings were severely stunted.

163. Grosso, John J., 1954, Control of tobacco blue mold by antibiotics. *Plant Disease Reporter* **38**, 333-337.

Initial experiments found streptomycin at 100 mcg/ml and zineb at 3 pound/100 gal. equally as effective in controlling tobacco blue mold. In artificial inoculation experiments streptomycin at 200 mcg/ml was significantly more effective than zineb at 3 pounds/100 gal.

164. Grove, Donald C. and Randall, William A., 1955, Assay Methods of Antibiotics. Medical Encyclopedia, Inc., New York. 238 pp.

A laboratory manual prepared by two scientists of the Antibiotics Division of the Food and Drug Administration. The book contains

biological and chemical assay methods for the detection of the more prominent antibacterial and antifungal antibiotics.

165. Hacker, Robert G. and Vaughn, John R., 1957, Cycloheximide analogues cause preinfection resistance to *Puccinia graminis* var. *tritici* in spring wheat. *Phytopathology* **47**, 14. Abstract.

The semicarbazone and oxime derivatives of cycloheximide appear to be systemic in their mode of action and exert a long-term effect. The oxime form was more effective than the semicarbazone when applied as a preinfection spray to wheat seedlings in the greenhouse.

166. Hacker, R. G. and Vaughn, J. R., 1957, Chemically induced resistance to stem rust of wheat by derivatives of actidione. *Plant Disease Reporter* **41**, 442-446.

The semicarbazone of cycloheximide at 50 mcg/ml induces preinfection resistance in spring wheat to black stem rust caused by *Puccinia graminis* var. *tritici*.

- 166a. Hacker, R. G. and Vaughn, J. R., 1958, Report on 1957 field tests of acti-dione derivatives for control of black stem rust of wheat. *Plant Disease Reporter* **42**, 609-613.

Further field tests of actidione "S" (the semicarbazone of the cycloheximide) for control of black stem rust of wheat confirm that this compound is extremely effective when used at high concentrations and rates.

167. Hamilton, J. M. and Szkolnik, M., 1953, Factors involved in the performance of cycloheximide (actidione) against *Cocomyces hiemalis*. *Phytopathology* **43**, 109. Abstract.

After a 96-hour incubation period cycloheximide at 2 mcg/ml was able to eradicate *C. hiemalis*, causal agent of cherry leaf spot. Sectioning of infected leaves showed that the pathogen had progressed to the palisade cells.

168. Hamilton, J. M., Szkolnik, M. and Sondheimer, E., 1956, Systemic control of cherry leaf spot fungus by foliar sprays of actidione derivatives. *Science* **123**, 1175.

Whereas cycloheximide is not particularly systemic in cherry, the oxime derivative was translocated a distance of 7-9 nodes. Translocation occurred at concentrations which were protective against subsequent inoculations.

169. Hampton, J. E., 1948, Cure of crown gall with antibiotics. *Phytopathology* **38**, 11-12. Abstract.

Galls caused by *A. tumefaciens* on bryophyllum, tomato, several species of *Prunus*, pear and other plants were cured with equal effectiveness by penicillin and streptomycin. The antibiotics were applied by immersion and hypodermic injection.

170. Havinga, E., Lynch, V., Norris, L. and Calvin, M., 1953, The effect of certain biologically active substances upon photosynthesis and dark CO₂ fixation. *Rec. des Trav. Chim.* **72**, 597-611.

Chlortetracycline and oxytetracycline influenced the CO₂ fixation in *Scenedesmus* as detected by C¹⁴ studies. Both compounds also accelerated the rate of photosynthesis from 2-6 times.

171. Hawthorne, M. E. and Wilson, G. B., 1952. The cytological effects of the antibiotic actidione. *Cytologia* **17**, 71-85,

Toxic effects of cycloheximide appear to be related to the prevention of normal cell organization during prophase and interphase of mitosis. Cycloheximide does not appear to destroy structures already present.

172. Heggsted, H. E. and Clayton, E. E., 1954, Control of tobacco wildfire with streptomycin sulfate. *Plant Disease Reporter* **38**, 661-665.

Severe infections of tobacco wildfire in the plant bed were eradicated by three weekly sprays at 200 mcg/ml.

173. Heggsted, H. E., Neas, M. O. and Grosso, John, 1956, Comparison of various streptomycin dust spray treatments for wildfire control in tobacco plant beds. *Plant Disease Reporter* **40**, 48-51.

In evaluating dusts and sprays of streptomycin for tobacco wildfire control, dusts of 3 and 6 pounds of 2,000 or 1,000 mcg/ml were compared with sprays of 5 and 10 gals. of 200 mcg/ml and the sprays were more effective.

174. Hemphill, D. D. and Goodman, R. N., 1955, Effects of plant-growth-regulating substances on control of *Erwinia amylovora* by streptomycin and terramycin. *Science* **122**, 122.

The improved disease control obtained by adding IAA to a streptomycin spray formulation was duplicated by additions of the ethyl ester of IAA, naphthyl acetamide and other non-indigenous growth regulators. The "growth regulator" effect is apparently not peculiar to IAA.

175. Hendricks, S. B., 1941, Base exchange of the clay mineral montmorillonite for organic cations and its dependence upon adsorption due to van der Waals forces. *Jour. Phys. Chem.* **45**, 65.

The adsorption of a number of organic bases to negatively charged silicate surfaces is influenced by van der Waals forces in addition to the electrical attraction of the organic cation for the negatively charged silicate surface.

176. Henry, A. W., Peterson, E. A., Millar, R. L. and Horricks, J. S., 1951. Control of covered smut of oats by seed treatment with an antibiotic, *Science* **113**, 390.

Oats naturally infested with covered smut were effectively decontaminated by cycloheximide at 10 mcg/ml but not by streptomycin at 1,000 mcg/ml.

177. Henry, A. W., Millar, R. L. and Peterson, E. A., 1952, Control of covered smut of wheat by rapid seed treatment with an antibiotic. *Science* **115**, 90.

Wheat seed infested with covered smut (*Tilletia* sp.) were completely protected from this pathogen by 0.5 and 10% cycloheximide dusts applied at the rate of $\frac{1}{2}$ to 1 oz/bu. These treatments were as effective as a 10 mcg/ml one minute dip.

178. Henry, A. W., Peterson, E. A. and Millar, R. L., 1953, Compatibility of actidione and orthocide in treatment of seed. *Phytopathology* **43**, 461.

Good control of covered smut of wheat was obtained with cycloheximide at 0.5 and 1.0% in a dust mixed with Dixie clay. The rate of application was $\frac{1}{2}$ oz/bu.

179. Herrell, W. E., 1950, Newer antibiotics. *Annual Review of Microbiology* **IV**, 101-128.

A brief review of the antibiotics considered to be new at that time. The paper includes such compounds as penicillin, streptomycin, chlortetracycline, oxytetracycline (had just been discovered), bacitracin, polymixin and bioercin.

180. Hervey, Ralph J., 1955, Stimulation of soil microorganisms by antibiotics. *Antibiotics and Chemotherapy* 5, 96-100.

Results from this study suggest that a number of factors influencing the microbial population of the soil may be involved in the phenomenon of antibiotic-induced plant growth stimulation.

181. Hessayon, D. G., 1953, Fungitoxins in the soil. II. Trichothecin: its production and inactivation in unsterilized soil. *Soil Science* 75, 395-404.

Small concentrations of trichothecin have a stimulatory effect upon the mycelial growth of *F. oxysporium* var. *cubense*, while higher concentrations have the usual inhibitory effect. Trichothecin is strongly adsorbed by soil, but little biological breakdown was detected.

182. Heuberger, J. W., Poulos, P. L. and Hood, J. J., 1950, Peach brown rot: liquid lime sulfur and certain chlorine compounds as post-harvest dip treatments for control in the package. *Phytopathology* 40, 12. Abstract.

Cycloheximide applied at 5-10 mcg/ml as a dip post harvest, for brown rot control, was extremely injurious to the fruit.

183. Heuber, J. W. and Poulos, P. L., 1953, Control of fireblight and frog-eye leaf spot (black rot) diseases of apples in Delaware in 1952. *Plant Disease Reporter* 37, 81-82.

An early report of fireblight control in the field. Streptomycin sulfate, thiolutin, oxytetracycline and copper rimocidin were used in this experiment. Streptomycin, thiolutin and the dithiocarbamates significantly reduced the incidence of this disease in apple.

184. Hildreth, R. C., 1957, The use of antibiotics for control of seed-borne bacterial blight of beans. IV International Congress of Crop Protection, Hamburg, Germany. Summary of paper 14, pp. 217.

Streptomycin seed treatments for bean blight affect germination by causing seed coat-slipping. This can be reduced by the addition of liquid latex to the seed-soak solution.

185. Hilborn, M. T., 1953, Effect of various chemicals on infection by *Rhizoctonia solani* and *Verticillium albo-atrum*. *Phytopathology* 43, 475. Abstract.

Rhizoctonia and *Verticillium* infections of potato stems were reduced considerably by a number of antibiotics including streptomycin, cycloheximide, polymixin, rimocidin and penicillin.

186. Holmes, F. W., 1955, Field and culture tests of antibiotics against *Graphium ulmi*. *Phytopathology* 45, 185. Abstract.

Of 22 antibiotics tested none were effective in controlling artificial inoculations of elm trees made with *Graphium ulmi*, causal agent of Dutch elm disease.

187. Hopwood, G. V., 1957, Antibiotics and plant protection. *Manufacturing Chemist*, Jan. 1957.

A review of recent advances in phytopathology with antibiotics.

188. Horner, C. E. and Maier, C. R., 1957, Antibiotics eliminate systemic downy mildew from hops. *Phytopathology* **47**, 525. Abstract.
Streptomycin sulfate and griseofulvin at high concentrations eliminated downy mildew from systemically infected hop shoots. Griseofulvin applied to basal shoots eliminated further production of systemically infected shoots for the remainder of the growing season.
189. Horsfall, James G., 1956, Principles of Fungicidal Action. Chronica Botanica Company, Waltham, Mass. 279 pp.
An exhaustive review and authoritative commentary on the principles of fungicidal action. Included are chapters on permeation into the fungus, disruption of cellular organization, effect on mitosis, morphology and growth, effect on metabolism of the fungus and others.
190. Howard, F. L. and Davies, M. E., 1953, *Curvularia* "fading-out" of turf grasses. *Phytopathology* **43**, 109. Abstract.
Cycloheximide was reported effective in controlling *Curvularia lunata* which causes "fading out" of turf grasses.
191. Huppert, M., MacPherson, D. A. and Cazin, J., 1953, Pathogenesis of *Candida albicans* infection following antibiotic therapy. I. The effect of antibiotics on the growth of *Candida albicans*. *Jour. of Bacteriology* **65**, 171-176.
Data suggest that the stimulation in growth of *C. albicans* is a result of the elimination of competing bacteria from the existing food supply.
192. Hutner, S. H. and Provasoli, L., 1955, In *Biochemistry and Physiology of Protozoa* **2**, 17-43. Hutner and Lwoff ed. Academic Press, Inc., New York. 388 pp.
The phytotoxic effect of streptomycin on higher plants is not upon chloroplasts once they are formed; rather the effect is upon the synthesis of chloroplast.
193. Iyengar, M. R. S. and Starkey, Robert L., 1953, Synergism and antagonism of auxin by antibiotics. *Science* **118**, 357-358.
Antibiotics are found to influence auxin action in two ways. Oxytetracycline and chloramphenicol increase the cell elongation promoted by IAA whereas citrinin reverses the antiauxin affect.
194. Jeffers, W. F., 1954, Use of actidione in culture of plant pathogenic bacteria. *Phytopathology* **44**, 144.
A method of culture for plant pathogenic bacteria is discussed. Cycloheximide is the antibiotic suggested for preparing fungus-inhibiting media.
195. Johnson, Leander F., 1957, Effect of antibiotics on the numbers of bacteria and fungi isolated from soil by the dilution plate method. *Phytopathology* **47**, 630-631.
Aside from streptomycin, the tetracyclines were most effective in inhibiting soil bacteria (*in vitro*). At low levels of chlortetracycline, 0.25-2.0 mcg/ml, significantly more fungal colonies developed than were observed in the streptomycin plates. It was postulated that either streptomycin inhibited the development of certain fungi or chlortetracycline stimulated their growth.
196. Katz, E. and Pienta, P., 1957, Decomposition of actinomycin by a soil microorganism. *Science* **126**, 402-403.

A bacterium of the genus *Achromobacter* destroyed up to 1,000 mcg/ml of actinomycin in 48 hours. This decomposition was believed to be enzymatic in nature.

197. Katznelson, H. and Sutton, M. D., 1951, Inhibition of plant pathogenic bacteria *in vitro* by antibiotics and quaternary ammonium compounds. *Canad. Jour. Bacteriology* **29**, 270-278.

An extensive *in vitro* evaluation of the activity of 6 antibiotics and several quaternary ammonium compounds against 33 species of phytopathogenic bacteria. Oxytetracycline and chlortetracycline were extremely effective; however, streptomycin, neomycin and polymixin were also highly active.

198. Kaufmann, M. J. and Chamberlain, D. W., 1957, The effect of antibiotics on *Pseudomonas glycinea*. *Plant Disease Reporter* **41**, 806-807.

The paper disc diffusion assay found the three tetracycline antibiotics active against *Pseudomonas glycinea*, the casual agent of bacterial blight of soybeans, at 10 mcg/ml and streptomycin at 100 mcg/ml. The latter was effective *in vivo* at 250 mcg/ml whereas the others were not. Manganese at 0.3% manganous sulfate successfully reversed the phytotoxic effect of streptomycin.

199. Ken Knight, Glenn, 1955, Chemotherapy of the peach rosette virus with antibiotics. *Phytopathology* **45**, 348 Abstract.

Lovell peach seedlings infected through bark-patch grafts with peach rosette virus were dipped in antibiotic solutions at 5 and 25 mcg/ml for 4 hours. Only the tetracycline antibiotics provided measurable degrees of recovery ranging from full to temporary. Streptomycin, endomycin, neomycin and cycloheximide were non-effective.

200. Keyworth, W. G., 1957, The use of streptomycin against silvering disease of red beet. *Annals Applied Biology* **45**, 215.

A 24-hour soak of red beet seed in 200 mcg/ml streptomycin reduced infection in plant beds from 1.0 to 0.025%. In a commercial plot of 4 acres, plants from untreated seed were 60% infected whereas the treated ones showed 0.1% diseased.

201. Kienholz, Jess R., 1955, Control of fireblight on Forelle pears with antibiotics at Hood River, Oregon. *Plant Disease Reporter* **39**, 208-209.

A 5-spray 100 mcg/ml streptomycin spray schedule for fireblight control initiated at near full bloom and continued at 10-day intervals effectively controlled the disease in the highly susceptible Forelle variety.

202. Kirby, R. S., 1954, Effectiveness of antibiotics for apple fire blight control under epidemic conditions in Penn. *Plant Disease Reporter* **38**, 432-433.

Comparing Agrimycin at 100 mcg/ml with pheno-lead for fireblight control, the former was extremely effective whereas the latter was ineffective.

203. Kirby, R. S., 1955, Control of tobacco wildfire with streptomycin preparations. *Plant Disease Reporter* **39**, 14.

In plant beds with 5-50% of the plants infected with *Pseudomonas tabaci*, 4 weekly sprays at 200 mcg/ml eradicated the disease. Further, in these plots tobacco blue mold was also controlled.

204. Kirby, R. S., 1957, Systox-lead arsenate, antibiotics, and sulfur in the control of powdery mildew on roses. *Plant Disease Reporter* **41**, 534-535.
Neither griseofulvin nor anisomycin at 100 mcg/ml was more effective than sulfur-ferbam or systox-lead arsenate combinations in controlling powdery mildew of roses.
205. Kirkpatrick, Hugh C. and Lindner, R. C., 1954, Studies concerning chemotherapy of two plant viruses. *Phytopathology* **44**, 529-533.
Chloramphenicol introduced into cucumber plants by vacuum infiltration markedly inhibited multiplication of (TMV) virus and (PLMV), the stone fruit virus. Assays performed were both biological and spectrophotometric.
206. Klemmer, H. W., Riker, A. J. and Allen, O. N., 1955, Inhibition of crown gall by selected antibiotics. *Phytopathology* **45**, 618-625.
Evidence presented suggest that oxytetracycline, chlortetracycline and chloramphenicol are at least partially inhibitory to the development of galled-cells of host tissue as well as the causal organism *Agrobacterium tumefaciens*.
- 206a. Klinkowski, M., 1948, Penicillin und streptomycin in der pflanzen-therapie. *Nachrichtenbl. f. d. Deutch. Pflanzenschutzd.* **NF 2** (7/8), 4 pp.
A review of the earliest efforts in plant pathology with antibiotics. The work discusses primarily the researches of Brown and co-workers and comments on penicillin production.
- 206b. Klinkowski, M., 1954, Die antibiotika und ihre bedeutung in der phyto-pathologie. *Sitzungsber. Deutsch. Akad. Landwirtschaftswiss. Berlin* **3** (17), pp. 1-35.
A review of investigations in plant disease control with purified antibiotics and crude culture filtrates. The author includes data from some of his own investigations wherein actidione and crude culture filtrates were evaluated for their activity against apple mildew (*P. leucotricha*). Under field conditions it was found that both actidione and the filtrates applied as foliar sprays significantly reduced the number of infections.
207. Klomparens, William, 1951, Toxicity of cycloheximide to certain wood-rotting fungi, and preliminary studies on absorption of the antibiotic by tissues. *Phytopathology* **41**, 22. Abstract.
A bioassay using *Poria microspora* on 2% malt extract was developed that permitted detection of cycloheximide at concentrations as low as 0.5 mcg/ml.
208. Koontz, H. and Biddulph, O., 1957, Factors affecting absorption and translocation of foliar applied phosphorus. *Plant physiology* **32**, 463-470.
Uptake of P^{32} when supplied as a foliar spray was superior to vein injections and the amount translocated increased as the concentration applied was increased. Glycerin increased absorption of P from KH_2PO_4 but reduced absorption from K_2HPO_4 . Surface active agents also reduced absorption.
209. Krasilnikov, N. A., Kuchaeva, A. G., Nikitina, N. I., and Soryabin, G. K., 1955, Microbial antagonists in plant diseases. 1st International Conference on Antibiotics in Agriculture, Washington, D. C., Oct. 19-21.

A compilation of results of Russian research with antibiotics in the area of plant disease control. The first investigations of this type were made in 1947. Some of the antibiotics employed were Mycetin, gramicidin, subtilin, streptomycin, penicillin, chlortetracycline and grizemin.

210. Krupka, L. R. and Crossan, D. F., 1955, Studies on the use of antibiotics for the control of bacterial spot of pepper. *Phytopathology* **45**, 465. Abstract.

A greenhouse study on absorption of streptomycin using the agar-diffusion method detected no activity in pepper leaves sprayed with 250 mcg/ml and washed shortly thereafter. Unwashed leaves demonstrated activity.

211. Krupka, L. R. and Crossan, D. F., 1956, Overwintering and control of *Xanthomonas vesicatoria*. *Phytopathology* **46**, 17-18. Abstract.

Artificially infested seed dipped for 5 minutes in 1 : 1000 HgCl₂, streptomycin at 200 mcg/ml and the water control revealed 0, 25 and 24% infected seedlings respectively.

212. Kutsky, R., 1952, Effects of indolebutyric acid and other compounds on virus concentration in plant tissue cultures. *Science* **115**, 19-20.

Oxytetracycline, streptomycin and subtilin were ineffective against TMV. However, indolebutyric acid and naphthaleneacetic acid reduced the virus concentration in tissue cultures.

213. Leben, C. and Keitt, G. W., 1947, The effect of an antibiotic substance on apple leaf infection by *Venturia inaequalis*. *Phytopathology* **37**, 14. Abstract.

A *Streptomyces* species was found active *in vitro* against 29 phytopathogenic fungi. A partially purified substance was inhibitory to growth of *Venturia inaequalis* at 1 : 8,000,000 and *Sclerotinia fructicola* at 1 : 11,000,000.

214. Leben, C. and Keitt, G. W., 1948, Greenhouse tests of an antibiotic substance as a protectant spray. *Phytopathology* **38**, 16. Abstract.

An impure antibiotic principle from a species of *Streptomyces* applied prior to inoculation with *V. inaequalis* afforded complete control of the disease. The material resisted leaching by precipitation and was partially effective when applied 4 days prior to inoculation.

215. Leben, C. and Keitt, G. W., 1948, An antibiotic substance active against certain phytopathogens. *Phytopathology* **38**, 899-906.

The antibiotic produced by a species of *Streptomyces* previously reported active against *V. inaequalis* was named Antimycin. This antibiotic is primarily antifungal; however, it is not active against the *Fusaria* species.

216. Leben, C. and Keitt, G. W., 1949, Laboratory and greenhouse; studies of antimycin preparations as protective fungicides. *Phytopathology* **39**, 529-540.

An excellent leaf disk assay for antimycin using *Colletotrichum circinans* as the test organism is described. This antibiotic is not particularly stable.

217. Leben, C., Stessel, G. J. and Keitt, G. W., 1951, An antibiotic that inhibits certain phytopathogens. *Phytopathology* **41**, 23-24. Abstract.

An active principle isolated from an unidentified species of *Streptomyces* was found to be active against all 16 fungi tested in the range of 0.47–150 mcg/ml.

218. Leben, C. and Keitt, G. W., 1952, Studies on helixin in relation to plant disease control. *Phytopathology* **42**, 168–170.

A partially purified preparation of helixin, an antifungal antibiotic, was tested as a protectant against tomato early blight. The LD-95 and LD-50 were 37.5 and 6.5 mcg/ml respectively. Helixin failed to demonstrate systemic qualities and was found inhibitory to the germination of some seeds at 25–100 mcg/ml.

219. Leben, C. and Fulton, R. W., 1952, Effect of certain antibiotics on lesion production by two plant viruses. *Phytopathology* **42**, 331–335.

An agar plate method for determining the inhibitory action of test organisms on lesion production of plant viruses is described. Results find streptothricin and oxytetracycline at 1000 mcg/ml inhibitory to tobacco necrosis and tobacco ring spot virus.

220. Leben, C., Arny, D. C. and Keitt, G. W., 1953, Small grain seed treatment with the antibiotic Helixin B. *Phytopathology* **43**, 391–394.

The activity of helixin B is discussed as seed disinfectant for oats and barley infected with *Helmenthosporium victorae* and *H. sativum*. The disease control afforded by this compound compared favorably with the mercurial Ceresan M. Using *Glomerella cingulata* it was found in tests on depression slides that helixin had an activity across an air space.

221. Leben, C., Arny, D. C. and Keitt, G. W., 1954, Effectiveness of certain antibiotics for the control of seedborne diseases of small grain. *Phytopathology* **44**, 704–707.

Methyl cellosolve, ethylene glycol and mixtures of ethylene glycol and Tween 20 were not as effective as ethanol as carriers for helixin B.

222. Leben, C. and Keitt, G. W., 1954, Effects of antibiotics in control of plant diseases. *Jour. of Agr. and Food Chem.* **2**, 234–239.

A review of some 84 research efforts with antibiotics in the area of plant disease control.

223. Leben, C. and Keitt, G. W., 1956, Phytotoxicity of antimycin A. *Antibiotics and Chemotherapy* **6**, 191–193.

The specific inhibitory effect of antimycin A upon young growing points of tomato plants was interpreted as being related to the inhibition of the cytochrome oxidase system. A component of this enzyme system is markedly inhibited by antimycin A.

224. Lechevalier, H., Acker, R., Corke, C., Haenseler, C. and Waksman, S., 1953, Candicidin, a new antifungal antibiotic. *Mycologia* **45**, 155–171.

From a group of 197 cultures of actinomycetes tested for activity against *Ceratostomella ulmi*, a strain of *Streptomyces griseus* yielded an antibiotic active against yeasts, yeast-like fungi and *C. ulmi*. Because of its activity against *Candida albicans* it was named candicidin.

225. Lemin, A. J. and Magee, W. E., 1957, Degradation of cycloheximide derivatives in plants. *Plant Disease Reporter* **41**, 447–448.

The uptake of cycloheximide acetate $2C^{14}$ in tomato plants was studied. The acetate form is absorbed by the roots; however, it is apparently translocated to the leaves as cycloheximide *per se*.

226. Leukel, R. W. and Webster, O. J., 1953, Sorghum seed-treatment test in 1953. *Plant Disease Reporter* **37**, 585.

Cycloheximide applied as a 1% dust to sorghum seed allowed 6.7% of the seed to become infected with covered kernel smut, caused by *Sphacelotheca sorghi*.

227. Leukel, R. W. and Mitchell, J. W., 1956, Smut control in sorghum with the antibiotic complex F-17. *Plant Disease Reporter* **40**, 1073.

Antibiotic complex F-17 applied as a dry dust to dry seed or moist seed or as a one hour soak provided practically complete disinfection of *S. sorghi* from sorghum seed.

228. Litwack, Gerald and Pramer, David, 1957, Absorption of antibiotics by plant cells. III. Kinetics of streptomycin uptake. *Archives of Biochemistry and Biophysics* **68**, 396-402.

The uptake of streptomycin by cells of the alga *N. clavata* fits monomolecular kinetic equations. The rate of uptake fits the Michealis-Menten equation. The energy of activation of this apparently ion binding transport system is 7,500 cal/mole.

229. Livingston, J. E., 1953, The control of leaf and stem rust of wheat with chemotherapeutants. *Phytopathology* **43**, 496-499.

Cycloheximide applied from the air appeared to have some value both as an eradicant and protectant against *Puccinia rubigo-vera* of wheat, causal agent of leaf and stem rust.

230. Lockwood, J. L., Leben, C. and Keitt, G. W., 1952, A culture plate for agar diffusion assays. *Phytopathology* **42**, 447.

A metal cooky pan with $\frac{1}{8}$ inch plate glass bottom and an asbestos cover make up a three-piece large antibiotic assay dish. Can be used to run a large number of samples with a built-in standard curve.

231. Lockwood, J. L. and Williams, Lansing E., 1956, Field experiments for control of bacterial wilt of sweet corn by antibiotic and Tween 20 sprays. *Plant Disease Reporter* **40**, 622-625.

Results indicate that systemic action of antibiotics may be involved in the reduction of bacterial wilt symptoms in corn. *B. stewartii* was more effectively controlled when plants were placed in a moist chamber for 24 hours after the antibiotics streptomycin and oxytetracycline were applied.

232. Lockwood, J. L., 1958, A method for studying absorption of streptomycin by using leaf disks of *Sedum purpureum*. *Phytopathology* **48**, 150-155.

Assays of juice from leaves sprayed with streptomycin gave values twice those of assays of leaf disks. It was also noted that leaves incubated in darkness absorbed as much streptomycin as those maintained in the light.

233. Logsdon, Charles E., 1957, The effect of certain antibiotics on potato production and ring rot control in Alaska. *Phytopathology* **47**, 22. Abstract.

Seed potatoes inoculated with bacterial ring rot treated with Agrimycin at 1,000 mcg/ml showed symptoms of the disease in 8% of the hills. The controls on the other hand showed symptoms in 39 per cent of the hills. Bacitracin was ineffective in these experiments.

234. MacKay, J. H. E. and Friend, J. N., 1953, The effectiveness of antibiotics against some bacterial plant pathogens. *Australian Journal of Biological Sciences* **6**, 481-484.

Six plant bacterial pathogens were observed for their sensitivity to a number of antibiotics. The tube dilution method was used and the antibiotics in order of their effectiveness were chlortetracycline, streptomycin, oxytetracycline, chloramphenicol, bacitracin and penicillin. *Corynebacterium michiganense* was the most sensitive of the bacteria studied.

235. Malcolmson, Jean F. and Bonde, Reiner, 1956, Studies in the control of bacterial and fungus decay of potato seed pieces. *Plant Disease Reporter* **40**, 708-713.

Good control of the fungus rots associated with streptomycin treatments were obtained with rimocidin, phygon, captan and zineb. These compounds did not appear to influence the effectiveness of streptomycin.

236. Marlatt, Robert B., 1955, Acti-dione sprays on cantaloupe. *Plant Disease Reporter* **39**, 824.

Cycloheximide sprays at 1, 2 and 4 mcg/ml applied with a sticker had no apparent phytotoxic effect upon cantaloupe plants.

237. Marlatt, Robert B., 1955, Effectiveness of streptomycin as a control for common bacterial blight of pinto bean. *Plant Disease Reporter* **39**, 213-214.

As many as 4 spray applications of streptomycin at 1,000 mcg/ml failed to control bacterial blight of pinto bean (*X. phaseoli*) in the Chino Valley area.

238. Marlatt, Robert B., 1956, Susceptibility of some vegetables to streptomycin injury. *Plant Disease Reporter* **40**, 200-201.

The sensitivity of 14 of the more common vegetable species to streptomycin sprays at concentrations up to 10,000 mcg/ml were studied. Potato and radish were more sensitive whereas pea, pepper and watermelon were the least sensitive.

239. Martin, J. P., 1950, Use of acid, rose bengal, and streptomycin in the plate method of estimating soil fungi. *Soil Science* **69**, 215-232.

The data presented suggest that crystal violet at 10 mcg/ml and streptomycin at 30 mcg/ml in Littman's oxgall agar is an excellent medium for determining total numbers of fungi without interference from bacterial contamination.

240. Martin, Mary and Gottlieb, D., 1952, The production and role of antibiotics in the soil. III. Terramycin and aureomycin. *Phytopathology* **42**, 294-296.

Although both oxytetracycline and chlortetracycline are amphoteric, they may be adsorbed by illite or bentonite clays. The adsorption is more tenacious on the bentonite expanding-lattice types.

241. Martin, Mary and Gottlieb, D., 1955, The production and role of antibiotics in the soil. IV. Activity of five antibiotics in the presence of soil. *Phytopathology* **45**, 407-408.

The polypeptide antibiotics, circulin, rimocidin, subtilin, and actinomycin at 500 mcg/ml were found to be inactivated by the colloidal fraction of soil. The basic antibiotic neomycin was similarly inactivated.

242. McClure, Thomas T. and Cation, Donald, 1951, Comparison of actidione with some other spray chemicals for control of cherry leaf spot in Michigan. *Plant Disease Reporter* **35**, 393-395.

Cycloheximide used in the nursery row was found to be effective at 1–2 mcg/ml in controlling both cherry leaf-spot and mildew. The material was judged more effective than Bordeaux mixture and less phytotoxic in that intense defoliation accompanying Bordeaux applications was not observed.

243. McClure, Thomas T., 1952, Experiences with cherry sprays in 1951. *Phytopathology* **42**, 14. Abstract.

Cycloheximide at 2 mcg/ml applied 3 times during the growing season gave good control of *C. hiemalis*, the causal organism of cherry leaf-spot.

244. Miller, H. N., 1956, A bacterial leaf rot of philodendron. *Phytopathology* **46**, 21. Abstract.

A bacterial soft rot of leaf and petiole of *Philodendron* is described. The causal organism appears to be of the genus *Erwinia* and the pathogen was adequately controlled with sprays of Agrimycin at 200 mcg/ml.

245. Miller, Patrick, 1957, Control by different streptomycin formulations of fire blight on Bosc pears in Connecticut. *Plant Disease Reporter* **41**, 19–22.

Streptomycin at 200 mcg/ml gave good control of field artificial inoculation of Bosc pears with *E. amylovora*. However, at 75 mcg/ml streptomycin plus additions of calcium nitrate, glycerine or kerosene failed to reduce the number of infections.

246. Miller, P. W. and Vaughan, E. K., 1957, Yellow virus complex in Marshall strawberry plants not inactivated by growth-regulating antibiotic, and certain other chemicals. *Plant Disease Reporter* **41**, 17–18.

Cycloheximide, Agrimycin, chlortetracycline, rimocidin and oligomycin, streptomycin and oxytetracycline were all unable to inactivate the type 2 virus of strawberry.

247. Mills, W. D., 1955, Fire blight development on apple in Western New York. *Plant Disease Reporter* **39**, 206–207.

A statistical treatment of temperature and precipitation data during apple bloom period from 1918–1945 revealed a) blossom blight was more prevalent when daily maximum temperature was 65°F or above with precipitation in bloom and b) a streptomycin spray program may be initiated once these prerequisites to infection, temperature and precipitation, are fulfilled.

248. Miric, Mirjana and Dunegan, John C., 1953, An antibiotic substance toxic to *Xanthomonas pruni* produced by *Aspergillus niger*. *Plant Disease Reporter* **37**, 157–158.

A thermostable (10 minutes at 110°C) antibiotic substance was found to be produced by *Aspergillus niger* which is active against *X. pruni*.

249. Mirzabekyan, R. O., 1952, The action on microbe antagonists and their antibiotic substances on a series of stimulators of bacteriosis in agricultural plants. *Review of Applied Mycology* **31**, 621–622. Abstract.

Three numbered antibiotics, 15, 15N, and 18, are reported to have some systemic activity against artificial inoculations with *B. armeniaca* and *X. malvacea* um. However, only antibiotic 15N was thought to penetrate all parts of the plant.

250. Mirzabekyan, R. O., 1955, Antibiotics as a measure for disinfecting apricot cuttings from internal infection. *Agrobiologia* **2**, 130–134.

Antibiotics grizemin and streptomycin at 1,000 mcg/ml applied as dips for 24 and 48 hours to apricot and peach cuttings eradicated established infections of *B. armeniaca*. Phytotoxic reactions were not observed from these treatments.

251. Mirzabekyan, R. O. and Menkova, K. A., 1955, Penetration and preservation of activity of antibiotics in plants in testing against phytopathogenic microorganisms. *Akad. Nauk SSSR. Izv. Ser. Biol.* **6**, 10-19.

Some antibiotics penetrate seeds and roots of plants and migrate to the above ground portions of plants. Penicillin appears to be the most rapidly moving antibiotic. The rate of movement appears to vary with both the antibiotic and the plant species.

252. Mitchell, J. W., Zaumeyer, W. J. and Anderson, W. P., 1952, Translocation of streptomycin in bean plants and its effect on bacterial blights. *Science* **115**, 114-115.

Translocation of streptomycin from an internodal application to primary and first trifoliate leaves was in sufficient quantity to control artificial inoculations of *P. phaseolicola*. Movement of streptomycin from foliage to pods through the peduncle did not appear to be extensive.

253. Mitchell, J. W., Zaumeyer, W. J. and Preston, W. H., 1953, Movement of streptomycin in bean plants. *Phytopathology* **43**, 480. Abstract.

Translocation of streptomycin and concomitant systemic protectant and eradicant action against halo blight is described. Translocation was upward in the stem and distal in the leaf.

254. Mitchell, J. W., Zaumeyer, W. J. and Preston, W. H., Jr., 1954, Absorption and translocation of streptomycin by bean plants and its effects on the halo and common blight organisms. *Phytopathology* **44**, 25.

The use of streptomycin dependent strain of *E. coli* established the fact that the antibiotic substance recovered from streptomycin treated leaves was in fact streptomycin and not a break-down product thereof.

255. Morgan, Billie S., Goldberg, Herbert S. and Goodman, Robert N., 1954-55, Residual quantities of a streptomycin-oxytetracycline combination, as determined by a simple microbiologic assay. *Antibiotics Annual 1954-55*, Medical Encyclopedia, Inc., New York, 536-539.

A serial dilution assay for streptomycin detection in plant tissue is described. Sensitivity of the assay using *E. amylovora* as a test organism was 0.3 mcg/ml and 0.15 mcg/ml when *K. pneumoniae* was used.

256. Morgan, Billie S. and Goodman, Robert N., 1955, *In vitro* sensitivity of plant bacterial pathogens to antibiotics and antibacterial substances. *Plant Disease Reporter* **39**, 487-490.

Species of the 5 major genera of plant bacterial pathogens were challenged with 8 antibiotics and 2 pyridine-thione compounds, *in vitro*. The tetracyclines, streptomycin and neomycin were the most active antibiotics evaluated. Except for a few *Pseudomonads*, these antibiotics inhibited the entire spectrum at concentrations lower than 1 mcg/ml.

257. Moss, Virgil D., 1957, Acti-dione treatment of blister rust trunk cankers on western white pine. *Plant Disease Reporter* **41**, 709-714.

Cycloheximide applied as a canker paint at 150 mcg/ml in combination with an isoparaffinic base oil was effective in eradicating juvenile pycnial and aecial cankers of western white pine blister rust.

258. Müller, K. O., MacKay, J. H. E. and Friend, J. N., 1954, Effect of streptomycin on the host-pathogen relationship of a fungal phytopathogen. *Nature* **174**, 878-879.

Tomato plants grown in solutions containing streptomycin resisted extensive infection from inoculations with *Phytophthora infestans*. Infection itself was not prevented but spread of the parasite was restricted after contact with the host plasma.

259. Murneek, A. E., 1952, Thiolutin as a possible inhibitor of fireblight. *Phytopathology* **42**, 57.

In this first successful field study utilizing antibiotics to control *E. amylovora*, the causal organism for fireblight of apple and pear, both streptomycin and thiolutin significantly reduced the number of infections.

260. Murneek, A. E., 1954, Antibiotics used in controlling fireblight outbreaks. *Better Fruit*, March, 6, 7, 30.

A review of progress in fireblight control with antibiotics.

261. Napier, E. J., Turner, D. I., Rhodes, A. and Toothill, J. P. R., 1956, The systemic action against *Pseudomonas medicaginis* var. *phaseolicola* of a streptomycin spray applied to dwarf beans. *Annals of Applied Biology* **44**, 145-151.

Streptomycin applied to primary leaves of dwarf beans exhibited a systemic antibacterial prophylactic action against *P. phaseolicola*. This effect was apparent at sites as far removed as the 4th trifoliate leaf.

262. Natti, John J. and Hervey, G. E. R., 1956, Influence of insecticide and fungicide sprays on downy mildew of broccoli. *Phytopathology* **46**, 242. Abstract.

Agrimycin at 1 pound per acre was the superior material in tests to control *Peronospora parasitica*, casual agent of downy mildew of broccoli.

263. Natti, John J., Hervey, G. E. R. and Sayre, C. B., 1956, Factors contributing to the increase of downy mildew of broccoli in New York state and its control with fungicides and agrimycin. *Plant Disease Reporter* **40**, 118-124.

In controlling *Peronospora parasitica* with Agrimycin at 1 pound per acre, it was found that this quantity applied in 25 or 100 gallons was equally effective. It was noted, however, that streptomycin injury, chlorosis, was more prevalent at the higher gallonage.

264. Natti, John J., 1957, Control of downy mildew of broccoli with antibiotics and fungicides. *Plant Disease Reporter* **41**, 780-788.

Cycloheximide, ayfactin and a number of other antibiotics were effective in inhibiting germination of *P. parasitica* spores but were ineffective *in vivo* in greenhouse tests. On the other hand streptomycin, mycostatin and anisomycin were only moderately successful in suppressing spore germination yet were particularly effective *in vivo*.

265. Natti, John J., 1957, Control of downy mildew of broccoli with antibiotics. *Phytopathology* **47**, 245-256. Abstract.

Streptomycin was the most effective antibiotic evaluated in controlling downy mildew of broccoli. The effectiveness of the antibiotic was enhanced by glycerol or by exposing the sprayed plants to constant misting. Streptomycin appeared to act as both a surface and internal protectant.

266. Nelson, Ray, 1951, Control of mint rust with dust fungicides. *Phytopathology* **41**, 27. Abstract.

In field experiments designed to control mint rust, caused by *Puccinia menthae*, a 2% cycloheximide dust proved to be less effective than ferbam but more so than zineb.

267. Nelson, Ray, 1951, Control of onion mildew with dust fungicides. *Phytopathology* **41**, 28. Abstract.

A 2% cycloheximide-sulfur dust was not particularly effective in controlling onion mildew, caused by *Peronospora destructor*, but did produce significant yield increases.

268. Newburgh, R. W. and Cheldelin, Vernon H., 1955, Effect of antibiotics on the growth of *Tilletia caries*. *Plant Disease Reporter* **39**, 684.

In vitro activity of a number of antibiotics at 0.025–2.0 mcg/ml against stinking smut of wheat was studied. Of the 10 antibiotics evaluated only oligomycin completely inhibited the fungus, *Tilletia caries*, and this was achieved at 0.4 mcg/ml.

269. Nickell, Louis G., 1952, Stimulation of plant growth by antibiotics. *Proc. Soc. for Exp. Biology and Med.* **80**, 615–617.

Data from 3 types of experiments show antibiotics to stimulate plant growth: a) tissue culture, b) seed germination and c) seed germination and subsequent growth in the soil.

270. Nickell, Louis G. and Finlay, Alexander C., 1954, Antibiotics and their effects on plant growth. *Journal of Agr. and Food Chemistry* **2**, 178–182.

Using *Lemna minor* grown under aseptic conditions it was found that oxytetracycline, bacitracin, penicillin and thiolutin increased growth as measured by gains in wet weight.

271. Nickell, Louis G., 1955, Effects of antigrowth substances in normal and atypical plant growth. *Antimetabolites and Cancer*, 129–151.

Stimulative effect of antibiotics on plant growth may be due to a) degradation products of the antibiotic, b) a chelation effect, c) detoxification of plant wastes, d) influence upon cell permeability, e) inactivation of a plant growth inhibitor and f) effects upon vitamin synthesis and hormonal balance.

272. Norman, A. G., 1955, The place of microbiology in soil science. *Advances in Agronomy* **7**, 399–410.

This paper is a philosophical one pertaining to the progress of research in soil microbiology. It includes the study of soil microbial populations and the application of information obtained from soil microbiology studies to the solution of problems in soil science.

273. Norman, A. G., 1955, The effect of polymyxin on plant roots. *Archives of Biochem. and Biophysics* **58**, 461–477.

The calcium ion is able to partially reverse the phytotoxicity of polymyxin to plant roots. It is suggested that both polymyxin and Ca^{++} are retained at similar adsorption sites on root surfaces. The adsorption of polymyxin is believed due to its purine-pyrimidine structures. The injury is caused by the surfactant action of the material.

274. Owgawa, Joseph M. and Vergara, Claudio, 1955, Effects of cycloheximide on powdery mildew of grapes and brown rot of peach fruits in the laboratory. *Phytopathology* **45**, 695. Abstract.

Plants sprayed with cycloheximide at 0.5 and 2.0 mcg/ml plus 1% each carbowax 4,000 and methyl cellosolve one day prior to inoculation with *Uncinula necator* were effectively protected against infection. Translocation of the antibiotic was not apparent; however, an eradicant potential for powdery mildew was noted.

275. Oort, A. J. P. and Dekker, J., 1957, Experiments with rimocidin and Fu 56, two fungicidal antibiotics with systemic action. IV. International Congress of Crop Protection, Hamburg, Germany. Paper 7, 214.

Rimocidin and Fu 56 are tetraene antibiotics which effectively disinfect seeds internally infected with *Ascochyta pisi*, *Colletotrichum lindemuthianum* and *Phoma betae*. Both antibiotics are translocated in bean plants from roots to leaves.

276. Palm, E. T. and Young, Roy A., 1957, The compatibility of certain organic fungicides and antibiotics in treatment mixtures as indicated by stability and phytotoxicity. *Plant Disease Reporter* **41**, 151-155.

Compatibility experiments showed dichlone and captan to be compatible with streptomycin and oxytetracycline. Streptomycin had no effect upon maneb, but oxytetracycline showed an immediate loss of activity when combined with this compound.

277. Paulus, Albert and Starr, G. H., 1951, Control of loose smut with antibiotics. *Agronomy Journal* **43**, 617. Abstract.

Of 8 antibiotics evaluated for control of loose smut of barley, none were completely effective. Streptomycin did, however, reduce the number of smutted plants 50%.

278. Petersen, D. and Cation, D., 1950, Exploratory experiments on the use of actidione for the control of peach brown rot and cherry leaf spot. *Plant Disease Reporter* **34**, 5-6.

This is the initial publication of field data demonstrating the efficacy of cycloheximide against *C. hiemalis*. This antibiotic was effective against cherry leaf spot at 1 mcg/ml but failed to suppress brown rot of peach caused by *Monilinia fructicola* at 20 mcg/ml.

279. Porter, R. H., 1954, Sorghum seed-treatment tests in Colorado. *Plant Disease Reporter* **38**, 88.

Cycloheximide applied as a slurry at the rate of 1 oz/bu of seed greatly reduced *sphacelotheca sorghi* infections. However, this treatment was not superior to a number of organic and inorganic mercurial compounds.

280. Pound, Glenn S. and Stahmann, Mark A., 1951, The production of a toxic material by *Alternaria solani* and its relation to the early blight disease of tomato. *Phytopathology* **41**, 1104-1114.

A crystalline toxin, apparently alternaric acid, produced by *A. solani* is phytotoxic to tomato at concentrations as low as .02 mcg/ml.

281. Powell, D. A., Adam, A. V. and Anderson, H. W., 1954, Relationship between timing of bactericidal applications and spring canker development on peaches. *Phytopathology* **44**, 502.

Neomycin applied at the rate of 2 oz/100 gallons compared favorably with copper sulfate in reducing the number of spring cankers in peach wood caused by *Xanthomonas pruni*.

282. Powers, H. R., Jr., 1957, Histology of wheat plants infected with the powdery mildew fungus (*E. graminis tritici*) and treated with anisomycin. *Phytopathology* **47**, 453. Abstract.

Anisomycin eradicated powdery mildew infections of wheat. Surface mycelia of the organism *E. graminis* var. *tritici* were sensitive to the antibiotic; however, haustoria penetrating the epidermal cells were not. It would appear that anisomycin is not sufficiently systemic to inactivate the fungus once penetration has been achieved.

283. Pramer, David and Starkey, Robert L., 1951, Decomposition of streptomycin. *Science* **113**, 127.

Streptomycin added to heat-sterilized soil at the rate of 1,000 mcg/ml lost no activity in a period of 3 weeks. In unsterile soil, however, more than $\frac{1}{2}$ of the activity disappeared in 1 week and a complete loss was noted at the end of 2 weeks. The addition of glucose or glutamic acid to unsterile soils delayed breakdown of streptomycin.

284. Pramer, David, 1953. Observations on the uptake and translocation of five actinomycete antibiotics by cucumber seedlings. *Annals of Applied Biology* **40**, 617-622.

Cucumber seedlings growing in 50 mcg/ml solutions of streptomycin showed accumulation against a concentration gradient since 90 mcg/ml were detected in the cotyledons and leaves. Chloramphenicol was also observed to be absorbed by these seedlings whereas the tetracyclines and neomycin were not detected.

285. Pramer, David, 1954, The movement of chloramphenicol and streptomycin in broad bean and tomato plants. *Annals of Botany* **18**, 72.

Neither chloramphenicol nor streptomycin accumulated against a concentration gradient in broad bean or tomato plants. Accumulation of chloramphenicol appeared to be a linear function of water uptake. The discriminatory ability of roots to limit antibiotic absorption as compared to cut shoot surfaces is discussed.

286. Pramer, David, 1955, Absorption of antibiotics by plant cells. *Science* **121**, 507-508.

The uptake of streptomycin by the algae *Nitella clavata* was influenced by temperature, a Q_{10} of 2.0 was calculated. This uptake which was against a concentration gradient was not affected by respiratory inhibitors.

287. Pramer, David and Wright, Joyce M., 1955, Some phytotoxic effects of five actinomycete antibiotics. *Plant Disease Reporter* **39**, 118-119.

Using the petri-dish seed germination technique the tetracyclines and chloramphenicol were more toxic than streptomycin or neomycin. Not only was germination reduced by all antibiotics, but root development and chlorophyll formation were also inhibited.

288. Pramer, David, 1956, Absorption of antibiotics by plant cells. II. Streptomycin. *Archives of Biochemistry and Biophysics* **62**, 265-273.

It is assumed that streptomycin accumulation is a cation exchange process requiring energy received from respiration. Data suggest that uptake is mediated by an ion binding carrier system, that specific absorption sites are involved and that metabolic poisons such as 2,4DNP inhibit the process.

289. Pramer, David, Robison, R. S. and Starkey, Robert L., 1956, The mode of action of antibiotics in the control of plant disease. *Phytopathology* **46**, 341-342.

The authors advance 4 possible modes of action by which antibiotics may suppress plant pathogens *in vivo*. Evidence is presented for all modes with exception of toxin neutralization.

- 289a. Pratt, R. and Dufrenoy, J. D., 1953, Antibiotics, J. B. Lippincott Company, 398 pp.

An excellent book on the subject of antibiotics. The authors have organized the subject under the following four parts: Fundamental aspects, Industrial aspects, Applied aspects and Modification of biologic and social systems.

290. Prescott, G. C., Emerson, Harold and Ford, J. H., 1955, Determination of cycloheximide (actidione) residues in cherries. *Ind. Microbiol. Soc. Proc.* 42. Abstract.

A bioassay for cycloheximide detection is described wherein sensitivity of the assay is 0.04 mcg/ml. With this method it was noted that the antibiotic has a half-life in cherry fruit of 24 hours. Enzymatic inactivation of the compound in cherry fruit is postulated.

291. Prescott, G. C., Emerson, Harold and Ford, J. H., 1956, Determination of cycloheximide (actidione) residues in cherries. *Jour. of Agr. and Food Chem.* 4, 343-345.

A chloroform extraction of cycloheximide from cherry fruit permits detection of this compound at 0.04 mcg/ml. The cylinder-plate agar diffusion method is described and the test organism is the yeast *S. pastorianus*.

292. Preston, W. H., Jr., Daly, E. J., Smale, B. C. and Mitchell, J. W., 1956, Effects of absorbed and translocated F-17 culture filtrate antibiotic factors on the bean rust antibiotic. *Phytopathology* 46, 469. Abstract.

Bean rust caused by *Uromyces phaseoli* var. *typica* is controlled by antibiotic F-17. The compound is translocated from upper surfaces to lower surfaces of bean leaves and is also absorbed from stems and moves upward to the primary leaves.

293. Pridham, T. G., Lindenfelser, L. A., Shotwell, O. L., Stodola, F. H., Benedict, R. G., Foley, C., Jackson, R. W., Zaumeyer, W. J., Preston, W. H., Jr. and Mitchell, J. W., 1956, Antibiotics against plant disease. I. Laboratory and greenhouse survey. *Phytopathology* 46, 568-575.

About 500 cultures of *Streptomyces* were screened for antibiotic activity by three methods against 12 phytopathogenic bacteria and fungi. Nine of these showed broad anti-fungal properties *in vitro* and against 1 fungus disease *in vivo*.

294. Pridham, T. G., Lindenfelser, L. A., Shotwell, O. L., Stodola, F. H., Benedict, R. G. and Jackson, R. W., Antibiotics against plant disease. II. Effective agents produced by a new strain of *Streptomyces cinnamoneus*. *Phytopathology* 46, 575-581.

Three separate fractions of an active filtrate of a *Streptomyces* sp. were found active against *U. phaseoli* *in vivo* as well as powdery mildew of beans and blue grass.

295. Pringsheim, Ernest G. and Pringsheim, Olga, 1952, Experimental elimination of chromatophores and eye-spot in *Euglena gracilis*. *New Phytologist* 51, 65-76.

Exposure of *Euglena gracilis* to streptomycin at 200-2,500 mcg/ml caused the cells to become apoplastidic (colorless). Apparently only certain strains of this algae are bleached by streptomycin. Strains which become colorless react similarly to high temperatures.

296. Rangaswami, G., 1954, Recent developments in the chemotherapy of plant diseases. *Madras Agricultural Jour.* **61**, 297-303.

A brief review of advances in plant chemotherapy up to 1954.

297. Rangaswami, G., 1956, A preliminary report on the use of mycothricin complex in plants. *Plant Disease Reporter* **40**, 483-487.

Mycothricin is apparently absorbed and translocated by wheat seed. The antibiotic was not toxic to wheat, tomato or cucumber seeds at 5,000 mcg/ml, their foliage at 2,500 mcg/ml or in liquid culture at 500 mcg/ml. An 18-hour dip at 2,500 mcg/ml completely suppressed growth of a number of fungal and bacterial pathogens present in and/or on wheat seed.

298. Rangaswami, G., 1956, *In vitro* effect of mycothricin on plant pathogenic bacteria and fungi. *Mycologia* **48**, 800-804.

Mycothricin, a basic antibiotic, was evaluated as an inhibitor of a number of plant pathogenic fungi and bacteria, *in vitro*. The most sensitive of 7 bacteria was *E. atroseptica* which was inhibited at 0.5 mcg/ml. The peach brown rot (*Monilinia fructicola*) fungus was inactivated at 2.5 mcg/ml.

299. Rangaswami, G., 1957, Development of resistance to streptomycin in *Xanthomonas citri* and *X. malvacearum*. *Cur. Sci.* **26**, 185-186.

300. Reilly, H. C., Schatz, A. and Waksman, S., 1945, Antifungal properties of antibiotic substances. *Jour. of Bacteriology* **49**, 585-594.

Of 7 antibiotics tested for their antifungal properties gliotoxin and streptothricin were the most effective. Three types of antifungal activity demonstrated by antibiotics are described: a) a lytic action, b) a fungicidal effect other than lysis and c) fungistasis or growth suppression. A strong fungistatic effect with limited host toxicity appears to be an essential characteristic of a good chemotherapeutant.

301. Rhodes, A., Crosse, R., McWilliam, R., Toothill, J. P. R. and Demar, A. T., 1957, Small plot trials of griseofulvin as a fungicide. *Annals of Applied Biology* **45**, 215-226.

Griseofulvin showed considerable promise in controlling *Botrytis* some powdery mildews.

302. Rhodes, G. N., Mullett, R. P. and Matthews, J. N., 1956, Results of wildfire test demonstration control treatments with streptomycin sulfate. *Plant Disease Reporter* **40**, 202-204.

As a preventive control for tobacco wildfire, streptomycin spray at 100 mcg/ml was superior to the standard copper drench. Eradication of infections of *P. tabaci* was effected with sprays at 200 mcg/ml. Some growth acceleration of algae was observed where streptomycin had been applied as a protectant.

303. Rich, Saul, 1954, Control of *Botrytis* sp. on stored roses. *Phytopathology* **44**, 503. Abstract.

Cycloheximide was ineffective in controlling *Botrytis* sp. on stored roses.

304. Rich, Saul, 1956, Seed treatments to protect corn seedlings against Stewart's Wilt. *Plant Disease Reporter* **40**, 417-420.

Corn seedlings developing from seed previously dipped in streptomycin and oxytetracycline were inoculated with *Bacterium stewartii*.

Wilt symptoms were significantly less severe in the treated seedlings than in the controls.

305. Rich, Saul, 1957, Griseofulvin, lithium salts, and zinc glass frit for control of cabbage club root. *Plant Disease Reporter* **41**, 1033-1035.

Griseofulvin applied as a soil treatment of 20-40 mg/kg of dry soil was effective in controlling cabbage club root caused by *Plasmidiophora brassicae*. When the antibiotic was applied to foliage no downward translocation was detected. At 40 mg/kg a slight stunting was observed.

306. Robbins, W. J., Hervey, Annette and Stebbins, Mary E., 1953, Euglena and vitamin B₁₂. *Annals New York Acad. Sci.* **56**, 818-829.

Evidence is presented that indicates that the action of streptomycin in producing chlorotic *Euglena* is not a selective one. The numbers of a given population which become bleached increase with increasing concentrations of streptomycin.

307. Robinson, R. S., Starkey, R. L. and Davidson, O. W., 1954, Control of bacterial wilt of chrysanthemums with streptomycin. *Phytopathology* **44**, 646-650.

Results of these experiments indicate that both streptomycin and oxytetracycline can be used to control bacterial wilt of chrysanthemums.

308. Rosen, W. G., 1954, Effects of streptomycin on certain green plants. *Ohio Jour. of Science* **54**, 75-78.

Chloroplast bleaching was studied in an attempt to discern the mode of action of streptomycin. Bleaching of bean foliage became more intense as the streptomycin concentration was increased. In addition to chloroplast bleaching streptomycin also induced root thickening, stem shortening, reduced root branching and suppression of leaf expansion.

309. Rosen, W. G., 1954, Plant growth inhibition by streptomycin and its prevention by manganese. *Proc. Soc. Exp. Biol. and Med.* **85**, 385-388.

The inhibitory effect upon growth caused by streptomycin could be prevented by 10^{-3} M of Mn⁺⁺. Competition for reaction sites is suggested as a possible explanation for this.

310. Rudolph, B. A., 1946, Attempts to control bacterial blights of pear and walnut with penicillin. *Phytopathology* **36**, 717-725.

In vitro tests showed both *E. amylovora* and *X. juglandis*, both gram negative bacterial pathogens, to be sensitive to comparatively high concentration of penicillin. At these concentrations, the antibiotic was bactericidal. *In vivo* experiments attempting to suppress these pathogens with penicillin were failures.

311. Rushdi, M. and Jeffers, W. F., 1956, Effect of some soil factors on efficiency of fungicides in controlling *Rhizoctonia solani*. *Phytopathology* **46**, 88-90.

This work suggests that the most important factor controlling the activity of antifungal substances in the soil is adsorption. This factor of adsorption is variously modified by pH change, soil type, organic matter and clay content of the soil. It was also noted that cycloheximide activity in the soil increased with increasing acidity.

312. Schlegel, D. and Rawlins, T. E., 1954, Inhibition of tobacco mosaic virus by antibiotic from actinomyceete, *Nocardia* sp. *Phytopathology* **44**, 328-329.

Antibiotic MK-61 produced by an actinomycete was found to have a direct inhibitory effect upon the TMV virus. Thus MK-61 is unlike many organic virus-inhibitors which affect the virus indirectly through some action upon the host's metabolism.

313. Schneider, I. R., 1957, Comparison of the effect of some antibiotics, antifungal substances, and Phenyl Carbamates on the growth of two vascular parasites *in vitro*. *Plant Disease Reporter* **41**, 436-441.

The *in vitro* activity of cycloheximide, gramicidin, endomycin, fungichromin, fradycin, ascocin and candicidin was evaluated against Dutch elm disease and Oak wilt. Fungichromin was the most active of these, inhibiting both pathogens completely at 20 mcg/ml. Ascocin and fradycin were also rather active.

314. Shaw, Luther, Lucas, G. B. and Throne, George F., Jr., 1957, Further studies with streptomycin alone and in combination with other chemicals for wildfire control in Burley tobacco plant beds, North Carolina, 1956. *Plant Disease Reporter* **41**, 99-102.

Effective control of tobacco wildfire was obtained with 6 weekly sprays of either streptomycin nitrate or sulfate. The addition of glycerin at 1% had only slight if any beneficial effect and the addition of tribasic copper decreased control.

315. Shaw, Luther and Lucas, G. B., 1957, Further studies with streptomycin for wildfire control in Burley tobacco plant beds, North Carolina, 1957. *Plant Disease Reporter* **41**, 939-940.

The application of streptomycin 2 or 3 times to tobacco plant beds 1 week after artificial inoculation with the wildfire bacterium, *Pseudomonas tabaci*, effectively eradicated the infection.

316. Silverman, W. B. and Hart, Helen, 1954, Antibiotics tested for control of wheat stem rust. *Phytopathology* **44**, 506. Abstract.

A number of antibiotics were applied at 500 and 600 mcg/ml in efforts to control *Puccinia graminis* inoculations effected 3 days previously. The antibiotics evaluated were streptomycin, dihydrostreptomycin, oxytetracycline, pleocidin, tyrothricin, neomycin, streptothricin, thiolutin and bacitracin.

317. Siminoff, P. and Gottlieb, D., 1951, The production and role of antibiotics in the soil: I. The fate of streptomycin. *Phytopathology* **41**, 420-429.

Using bentonite and illite 2 : 1 crystal-lattice structure clays, it was found that the expanding lattice or bentonite type adsorbed more streptomycin than the illite or fixed lattice type. Through evidence obtained from base exchange experiments it was shown that all but 5% of the streptomycin adsorbed by the bentonite clay is irreversibly bound. Streptomycin apparently acts as a trivalent cation with a Zeta potential.

318. Skiver, R. E., 1956, The use of antibiotics and fungicides in mist propagation. *Plant Disease Reporter* **40**, 1074-1080.

A unique method of applying antibiotics to cuttings in propagating beds is discussed. Antibiotics were introduced through an intermittent mist system which operated 4-5 seconds in each 60 second cycle. The antibiotics used were cycloheximide, streptomycin and tomatine. They were applied to carnation cuttings and all three materials reduced rooting of the cuttings.

319. Slack, D. A. and Hamblen, M. L., 1957, Control of current-season infection of peach by *Xanthomonas pruni* in Arkansas. *Phytopathology* 47, 33. Abstract.

The application of streptomycin as a foliar spray to Elberta peach partially controlled foliage infection. In most instances severity of infection was reduced by one-half where streptomycin was used at 100 mcg/ml. Fruit infections were also reduced by approximately 50%.

320. Smith, Wilson L., Jr., 1955, Streptomycin sulfate for the reduction of bacterial soft rot of package spinach. *Phytopathology* 45, 88-90.

Streptomycin applied as either pre-harvest sprays or post-harvest dips reduced soft rot to a point where decay was not evident after 2 days of storage at 70°F. Post-harvest dips were slightly more effective than the pre-harvest sprays.

321. Stessel, G. J., Leben, C. and Keitt, G. W., 1953, Partial purification and properties of the antifungal antibiotic, toximycin. *Phytopathology* 43, 23-26.

The antibiotic toximycin and its production are discussed. *In vitro* activity against 20 fungi and 3 bacteria ranged from 6.25-100 mcg/ml. The E.D. 50 and 95 for *Alternaria solani* in greenhouse experiments were 44 and 300 mcg/ml respectively. The antibiotic is produced by *B. subtilis* and is a polypeptide.

322. Stoddard, E. M., 1957, A *Fusarium* rot of geraniums and its control. *Plant Disease Reporter* 41, 536.

A *Fusarium* rot of geranium and a bacterial rot caused by a *Xanthomonas* were both excellently controlled by 5 weekly drenches with oxyquinoline sulfate at 250 mcg/ml and streptomycin at 200 mcg/ml. The two materials complement each other but do not show the adjuvant effect of the copper-streptomycin combinations.

323. Stokes, Anne, 1954, Uptake and translocation of griseofulvin by wheat seedlings. *Plant and Soil* V, 132-142.

Griseofulvin applied to wheat seedlings at 5-20 mcg/ml appeared in the guttation water 2 days after treatment. Concentration of the antibiotic in the guttation water was proportional to the concentration applied. Concentration in the guttation water was influenced by respiratory poisons such as 2,4-DNP and sodium azide and appears also to be related to transpiration rate.

324. Stoller, B. B., West, R. E. and Bailey, J. F., 1956, Controlling the mildew disease of the cultivated mushroom. *Plant Disease Reporter* 40, 193-195.

Of three antibiotics studied, cycloheximide, fradecin and oxytetracycline, only cycloheximide at 200 mcg/ml was able to inactivate the mushroom mildew, caused by *Dactylium dendroides*.

325. Strong, Forrest C. and Klomparens, William, 1955, The control of rust on cedar-apple and hawthorn with acti-dione. *Plant Disease Reporter* 39, 569.

Cycloheximide at 25 and 50 mcg/ml formulated in 0.5% summer Casco oil and applied after telial horns of cedar apple rust were formed inhibited the formation of sporidia.

326. Sutton, M. D. and Bell, W., 1954, The use of aureomycin as a treatment for swede seed for the control of black rot (*Xanthomonas campestris*). *Plant Disease Reporter* 38, 547-552.

Chlortetracycline applied as a 30-minute dip to swede seed reduced to zero black rot infections caused by *X. campestris* whereas the controls were 58% infected. Activity of the antibiotic was detected in stored seed for as long as 9 months after treatment.

327. Swartwout, H. G., 1955, Preliminary trials with acti-dione against powdery mildew on roses, cedar rust on apples, and downy mildew on grapes. *Plant Disease Reporter Sup.* 234, 131.

Concentrations of cycloheximide as low as 1 and 2 mcg/ml eradicated powdery mildew on leaves and buds of roses. Downy mildew of grapes was almost completely inhibited by this antibiotic at 1 mcg/ml. Similar concentrations arrested the development of cedar rust lesions on apple leaves.

328. Szkolnik, Michael and Hamilton, J. M., 1957, Control of peach leaf curl with omadine and of brown rot with omadine and certain antibiotics. *Plant Disease Reporter* 41, 29-292.

Post-harvest dip treatments of peaches artificially inoculated with the brown rot organism *Sclerotinia fructicola* were affected with fungichromin, mycostatin, and oligomycin. All gave excellent control at 200 mcg/ml whereas the standard fungicides captan and micronized sulfur were non-effective.

329. Tanaka, N. and Sato, S., 1952, Effects of streptomycin on the mitotic cells of *Tradescantia paludosa*. *Cytologia* 17, 124-133.

The action of streptomycin upon mitotic cells of *Tradescantia* root tips was studied cytologically using 25-1,000 mcg/ml concentrations. Every kind of cytological disruption, comparable to those inducible by x-ray or mutagenic chemicals, was observed e.g., clotting of chromosomes, stickiness of chromosomes, contraction of chromosomes, fragmentation and reductional grouping. These observations indicate that the action of streptomycin on mitotic cells is mutagenic.

330. Tarjan, A. C. and Howard, F. L., 1953, Comparison of benzothiazolyl-2-thioglycollic acid derivatives with other chemicals for Dutch elm disease therapy. *Phytopathology* 43, 486. Abstract.

Cycloheximide was found to be ineffective as either a protectant or eradicator of *Ceratostomella ulmi*, the causal agent of the Dutch elm disease.

331. Thanos, Andrew, 1952, Effect of cycloheximide (actidione) and some environmental factors on the germination of spores of *Monolinia fructicola* and *Botrytis cinerea*. *Phytopathology* 42, 21. Abstract.

Such factors as temperature and pH affect the activity of cycloheximide in inhibiting spore germination.

332. Therumalachar, M. J., Patel, M. K., Kulkarni, N. B. and Dhande, G. W., 1956, Effects *in vitro* of some antibiotics on thirty-two *Xanthomonas* species occurring in India. *Phytopathology* 46, 486-488.

Thirty-two *Xanthomonas* species were found to be sensitive to chlortetracycline at 160 mcg/ml or less, oxytetracycline at 60 mcg/ml or less and dihydrostreptomycin at 20 mcg/ml. The activity of penicillin against those species was not detected until 250 mcg/ml were employed.

333. Timonin, M. I., 1946, Activity of patulin against *Ustilago tritici*. *Scientific Agriculture* 26, 358-368.

The activity of patulin, a metabolic by-product common to a number of fungal species, e.g., *Penicillium patulum*, *P. claviforme* and *A. clavatus* is described. Activity against species of *Aschochyta*, *Epidermaphyton* and *Trichophyton* at 50 mcg/ml and against *Ustilago tritici* at 10 mcg/ml was observed. The antibiotic was inactivated by compounds containing sulphydryl groups.

334. Tukey, H. B., 1948, A note on the fungicidal property of actidione. *Science* **108**, 664.

Attention is called to the excellent fungicidal properties of cycloheximide.

335. Van Schaak, V., 1948, Antibiotics and potato ring rot. *Phytopathology* **38**, 27. Abstract.

In vitro experiments showed potato ring rot caused by *Corynebacterium sepedonicum* to be sensitive to both penicillin and streptomycin. However, *in vivo* experiments found only streptomycin to be effective in suppressing the disease in artificially inoculated seed pieces.

336. Vaughn, John R., Lockwood, John L., Randwa, G. S. and Hammer Charles, 1949, The action of actidione on plant tissue and upon certain fungi. *Michigan Agricultural Experiment Station Quarterly Bulletin* **31**, 456-464.

Cycloheximide used as a seed dip applied at various concentrations for 4 hours delayed or inhibited the germination of wheat, radish and bean seeds. Pea seeds which absorbed less water than other species were less severely affected.

337. Vaughn, John R., 1951, Cycloheximide, an antibiotic effective against turf diseases. *Phytopathology* **41**, 36. Abstract.

Sprays of cycloheximide at 200 mcg/ml applied every 2 weeks were more effective in controlling *Sclerotinia homeocarpa* and "melting out" caused by a *Helminthosporium* sp. than 2 standard turf fungicides.

- 337a. Van Overbeek, J., 1956, Absorption and translocation of plant growth regulators. *Annual Review of Plant Physiology* **7**, 355-372. Annual Reviews, Inc., Palo Alto, California.

The subject of absorption, permeation and translocation of organic substances by growth regulators is approached from such aspects as structure of the cuticle, chemistry of the cuticle, penetration into leaves structure and properties of the plasma membrane. Also discussed was polar transport.

338. Vaughn, John R. and Klomparens, W., 1952, Comparison of cycloheximide (acti-dione) with ten other fungicides for the control of turf diseases. *Phytopathology* **42**, 22.

An evaluation of cycloheximide and 10 other fungicides for the control of turf diseases. "Melting out" and "dollar spot" were excellently controlled by cycloheximide.

339. Visor, F. C., Carroll, V. J. and O'Neill, E. F., 1955, Use of antibiotic against agricultural plant pathogens. *Antibiotics Annual* **2**, 540-543.

Seven phytopathogenic bacteria were challenged with streptomycin and oxytetracycline alone and in a 10 : 1 ratio. The data indicate that the combination is more effective *in vitro* than streptomycin alone. Under the conditions of these experiments the combination was "definitely synergistic."

340. Von Euler, H., 1947, Nukeleinsäuren als wuchstaffe in gegenwart von colchicin und von streptomycin. *Arkiv f. Kemi. Mineral och Geol.* **24A**, 1-9.

Streptomycin was observed to have certain profound effects upon germinating seeds of rye, barley and cress. First, a general or non-specific growth inhibition expressed as a root thickening and coleoptile stunting was observed. The second effect was a chlorophyll bleaching which was induced by an exposure of these seeds to 0.2% streptomycin.

341. Vörös, J., Kiraly, Z. and Farkas, G. L., 1957, Role of polyphenolase in streptomycin-induced resistance to *Phytophthora* in potato. *Science* **126**, 1178.

This study pertains to the mechanism of streptomycin action *in vivo*. It was demonstrated that streptomycin absorbed by potato tissues greatly enhances their polyphenolase activity. It was proposed, therefore, that both natural and streptomycin-induced resistance of potato to *Phytophthora infestans* depend upon the same biochemical mechanism.

342. Waggoner, Paul E., 1956, Chemical treatment of potato seed in Connecticut 1955. *Plant Disease Reporter* **40**, 411-413.

An *in vitro* experiment found the activity of streptomycin against *E. carotovora* not affected by either captan or rimocidin and, conversely, the effectiveness of rimocidin and nabam against *Fusarium samblicinum*. It was further found that streptomycin did not increase (stimulate) the growth of *Fusarium in vitro* but did increase the susceptibility of the potato slices to the fungus.

343. Waksman, S. A., Bugie and Rielly, H. C., 1944, Bacteriostatic and bactericidal properties of antibiotic substances with special reference to plant pathogenic bacteria. *Bulletin Torrey Botanical Club* **71**, 107-121.

Plant pathogenic bacteria were found not to vary greatly in their sensitivity to antibiotic substances from other bacteria. Variations due to source were not as great as variations between species.

344. Walker, J. C. and Stahmann, M. A., 1955, Chemical nature of disease resistance in plants. *Annual Review of Plant Physiology* **6**, 351-366.

From this review of the literature, specifically of histological studies, it is apparent that resistance is not generally associated with histological differences. This would suggest that biochemical or physiological differences are more directly related to the phenomenon termed resistance.

Many of these biochemical and physiologic factors are discussed herein.

345. Wallen, V. R., Sutton, M. D. and Skolko, A. J., 1950, The effect of actidione on the growth of certain pathogenic fungi and on the germination of pea seed. *Phytopathology* **40**, 156-160.

In a series of *in vitro* experiments cycloheximide was found to be completely inhibitory to *Ascochyta pisi* and *Colletotrichum lindemuthianum* at 1 mcg/ml. Peas internally infected with *A. pisi* were dipped for 24 hours in cycloheximide at 1, 14, 40 and 225 mcg/ml. The fungus was not controlled, but germination was inhibited in direct proportion with the concentration of the antibiotic.

346. Wallen, V. R., 1955, Control of stem rust of wheat with antibiotics. I. Greenhouse and field tests. *Plant Disease Reporter* **39**, 124-127. Abstract.

Control of wheat stem rust (*Puccinia graminis*) with cycloheximide, gliotoxin, trichothecin, candicidin, endomycin and helixin B was attempted in greenhouse experiments. Of these materials only cycloheximide was effective.

347. Wallen, V. R. and Bell, W., 1956, Treatment of vegetable seed for improved emergence 1955. *Plant Disease Reporter* **40**, 129-132.

Improved emergence of cucumber, vegetable marrow and muskmelon was obtained from seed treatments with filipin.

348. Wallen, V. R. and Millar, R. L., 1957, The systemic activity of cycloheximide in wheat seedlings. *Phytopathology* **47**, 291-294.

The systemic activity of cycloheximide in wheat seedlings was demonstrated by bioassay procedures. Cycloheximide applied as a spray to wheat foliage was recovered from leaf tissue for at least 5 weeks after application. Translocation of the material was proved by detecting it in foliage after being applied to roots of seedlings growing in quartz sand or soil.

- 348a. Wallen, V. R., 1958, Control of stem rust of wheat with antibiotics. II. Systemic activity and effectiveness of derivatives of cycloheximide. *Plant Disease Reporter* **42**, 363-366.

Antibiotic activity of the oxime, acetate and semi-carbazon derivatives of cycloheximide was detected in foliage of which seedlings supplied these chemicals while the plants were grown in quartz sand. The acetate and semi-carbazon showed a greater systemic activity than the oxime.

349. Weindling, R. and Emerson, O. H., 1936, Isolation of a toxic substance from the culture filtrate of *Trichoderma*. *Phytopathology* **26**, 1068-1070.

The isolation of an antibiotic substance produced by *Trichoderma lignorum* is described. The substance known as gliotoxin was lethal to *Rhizoctonia* hyphal growth at one-tenth this concentration.

350. Weindling, R., Katznelson, H. and Purdy, H. B., 1950, Antibiosis in relation to plant diseases. *Annual Review of Microbiology* **4**, 247-260.

A discussion of antibiotics as related to plant diseases. A brief review of work done with antibiotics *per se* against plant pathogens *in vitro* and *in vivo* is also presented.

351. Wells, Homer D., and Robinson, B. P., 1954, Cottony blight of ryegrass caused by *Pythium aphanidermatum*. *Phytopathology* **44**, 509-510.

Cycloheximide showed promise in controlling cottony blight of ryegrass caused by *Pythium aphanidermatum*.

352. Whiffen, Alma J., Bohonos, N. and Emerson, R. L., 1946, The production of an antifungal antibiotic by *Streptomyces griseus*. *Journal of Bacteriology* **52**, 610-611. Abstract.

The initial report on cycloheximide, an antibiotic produced by *Streptomyces griseus* which also produces streptomycin. The spectrum presented suggests this antibiotic to be a strong yeast inhibitor.

353. Whiffen, Alma J., 1950, The activity *in vitro* of cycloheximide (actidione) against fungi pathogenic to plants. *Mycologia* **62**, 253-258.

The inhibitory effect of cycloheximide against fungi *in vitro* is discussed. Activity is displayed against a wide variety of pathogens including 1 Phyeomycete, 4 Ascomycetes, 1 Basidiomycete and 12

genera of the Fungi Imperfecti. For the 33 species studied the minimum inhibitory concentration (MIC) varied from 0.125–100.0 mcg/ml.

354. Williams, Lansing E. and Lockwood, John L., 1956, Control of bacterial wilt of cucumber by antibiotic sprays. *Plant Disease Reporter* **40**, 479–482.

Both the leaf-abrasion and stem puncture were evaluated as methods of artificially inoculating cucumbers with *Erwinia tracheiphila*. The hypodermic puncture was found more suitable and in addition it was observed that neomycin controlled leaf-rub inoculations but failed to suppress infections established by hypodermic stem puncture. Streptomycin and oxytetracycline were also studied and of the 3, streptomycin was superior.

355. Williams, Lansing E. and Lockwood, John L., 1957, Effect of antibiotics and surface-active agents on bacterial wilt of sweet corn in the greenhouse. *Phytopathology* **47**, 44–48.

Corn plants were sprayed with 4 antibiotics and 9 surface-active agents. Twenty-four hours later these plants were challenged with a hypodermic injection of *Bacterium stewartii*. Cycloheximide was found phytotoxic and streptomycin, oxytetracycline and penicillin not particularly effective. The first two were more effective if plants sprayed were kept in a moist chamber for 24 hours after spraying. Development of bacterial wilt of corn was partially inhibited by most of the surfactants at 10,000 mcg/ml.

356. Williams, Lansing E., 1957, Effects of some materials on Stewart's bacterial wilt of sweet corn when applied as seed treatments. *Plant Disease Reporter* **41**, 919–922.

Streptomycin, oxytetracycline, tetracycline, IAA, 2,4,5-trichlorophenoxyacetic acid and sodium borate applied in water solutions to corn seed as a 24-hour soak reduced the severity of *B. stewartii* symptoms.

257. Winter, H. F. and Young, H. C., 1953, Control of fireblight of apples in Ohio in 1953. *Plant Disease Reporter* **37**, 463–464.

Apple trees were inoculated in the field with fireblight (*E. amylovora*) and subsequently sprayed twice with streptomycin, oxytetracycline and zineb. One spray was applied prior to inoculation. Streptomycin was most effective, followed closely by oxytetracycline with zineb a very poor third.

358. Winter, H. F. and Young, H. C., 1954, Streptomycin most effective in controlling fireblight of apple and pear. *Phytopathology* **44**, 511. Abstract.

Streptomycin applied to apples in early bloom, full bloom and petal fall gave excellent control of fireblight on Jonathan apple and Bartlett pear. Concentrations which were found effective ranged from 50–120 mcg/ml.

359. Winter, H. F. and Young, H. C., 1955, Comparative studies on control of fireblight in apple and pear. *Jour. of Agr. and Food Chem.* **3**, 623–624.

A review of laboratory, greenhouse and field experiments which indicate streptomycin to be effective in controlling fireblight. Both streptomycin and oxytetracycline were more effective protectants for

artificially inoculated apple twigs if these twigs were maintained in a cellophane bag. This condition approximated a miniature moist chamber.

360. Wolley, D. W., Schaffner, G. and Braun, Armin C., 1955, Studies on the structure of the phytopathogenic toxin of *Pseudomonas tabaci*. *Journal of Biological Chem.* **215**, 485-493.

The phytopathogenic toxin of *Pseudomonas tabaci* is shown to be a lactone and is structurally analogous to methionine. Previous work has shown that methionine is a specific antagonist of the toxin. As a result it is postulated that the toxin is a naturally occurring anti-metabolite of methionine.

361. Wooliams, G. E., 1957, Bean halo blight control with streptomycin sulfate. *Phytopathology* **47**, 538. Abstract.

In a replicated seed-treatment experiment, diseased bean seeds were soaked for 30 minutes prior to planting in streptomycin solutions at concentrations of 50, 100, 250 and 500 meg/ml. Average halo blight incidence was 56, 49, 36 and 38% respectively as compared with 72% for the untreated control.

362. Wright, J. M., 1951, Phytotoxic effects of some antibiotics. *Annals of Botany* **15**, 493-499.

The toxicity of a number of antibiotics as well as coumarin and IAA to wheat, clover and mustard was assessed by a simple germination test. Wheat germination was not inhibited by the compounds tested, but those that were showed a concomitant reduction in root growth. The most toxic antibiotics were alternaria acid, glutinosin, mycophenolic acid and gliotoxin. Streptomycin was among the less toxic as were griseofulvin and penicillin. At high concentrations streptomycin appeared to inhibit chlorophyll synthesis.

363. Yarwood, C. E., 1956, Obligate parasitism. *Annual Review of Plant Physiology* **7**, 115-142.

When one organism can grow only by securing its food from continued association with another living organism the condition is termed obligate parasitism. This subject of obligate parasitism is wonderfully reviewed by the author who presents the problem of obligate parasitism from a theoretical point of view. The position is also taken that "obligates" will eventually be cultured.

364. Young, William J. and Beneke, E. S., 1952, Treatments to prevent fruit storage rots. *Phytopathology* **42**, 24. Abstract.

Cycloheximide at 2 meg/ml was of no value in controlling molds or in preserving appearances.

365. Young, William J. and Fulton, Robert H., 1951, A field test of several fungicides for the control of powdery mildew on *Lucretia* dewberry. *Plant Disease Reporter* **35**, 540-541.

Cycloheximide applied twice as a spray at 2 meg/ml was found to be effective as a protectant against *Sphacrotheca humuli*. This regime was not effective as an eradicant. It was also observed that the addition of Triton B1956, a surfactant, increased the efficacy of cycloheximide as a protectant.

366. Zaumeyer, W. J. and Fisher, H. H., 1953, Field control of halo blight of beans with streptomycin. *Phytopathology* **43**, 407.

A spray schedule using streptomycin at 100 mcg/ml from 1-4 times in bean plantings inoculated in the field with halo blight, *Pseudomonas phaseolicola*, showed 41, 10, 0 and a trace per cent infection where 1, 2, 3 and 4 sprays were applied. The controls were 93% infected and there were some 700 plants per plot.

367. Zaumeyer, W. J., 1955, Antibiotics and plant health. *Jour. of Agr. and Food Chem.* **3**, 112-116.

A general review of 18 researches pertaining predominantly to the use of antibiotics for the control of vegetable crop diseases.

368. Zaumeyer, W. J. and Wester, R. E., 1956, Control of downy mildew of lima beans with streptomycin. *Phytopathology* **46**, 32. Abstract.

At a concentration of 100 mcg/ml, 3 commercial streptomycin formulations protected lima bean plants from artificial inoculations of *P. phaseoli*. These formulations were superior to pure streptomycin at 150 mcg/ml. A streptomycin dust at 1,000 mcg/ml was as effective as a spray at 100 mcg/ml. A streptomycin dust at 500 mcg/ml was ineffective.

369. Zaumeyer, W. J. and Wester, R. E., 1956, Control of several fungus diseases of beans and lima beans with antibiotics. *Phytopathology* **46**, 470. Abstract.

Lima beans sprayed with streptomycin-containing formulations at 100 mcg/ml were almost completely protected from artificial inoculations of downy mildew (*Phytophthora phaseoli*). Pure streptomycin was not as effective as the more crude formulations, suggesting that impurities in some way improved the efficacy of streptomycin. Addition of copper to streptomycin appeared to be synergistic whereas the addition of glycerol proved non-beneficial.

370. Zaumeyer, W. J. and Wester, R. E., 1956, Control of downy mildew of lima beans with streptomycin. *Plant Disease Reporter* **40**, 776-780.

A report on greenhouse evaluations of the efficacy of fungicidin, oligomycin, griseofulvin, filipin and anisomycin against a number of plant fungal pathogens. All antibiotics were active against 1 or more of the 4 pathogens studied, *Uromyces phaseoli*, *Phytophthora phaseoli*, *Colletotrichum lindmuthianum* and *C. truncatum*.

371. Zaumeyer, W. J., 1956, Improving plant health with antibiotics. First International Conference on Antibiotics in Agriculture publication 397. National Research Council, Washington, D. C.

A comprehensive review of antibiotics in plant disease control, undoubtedly the most complete survey of the literature to that time. Areas of investigation are covered by major crop divisions as well as a discussion on mode of action, research progress and goals for future investigations.

372. Zaumeyer, W. J. and Doolittle, S. P., 1956, Control of late blight on tomato with streptomycin. *Phytopathology* **46**, 32. Abstract.

Three commercial streptomycin-containing formulations applied at 100 mcg/ml, 24 hours prior to inoculation, with a spore suspension of *Phytophthora infestans* protected tomato seedlings from infection. Incubation was for 48 hours in a fog chamber. The disease rating was 0.4 for the treatments and 4.0 for the control.

373. Zaunmeyer, W. J., 1957, Comparative protection of bean leaves from fungus infection by antibiotic treatments of lower and upper surfaces. *Phytopathology* **47**, 539. Abstract.

Treating lower surfaces of bean leaves with either oligomycin or anisomycin protected upper surfaces more effectively against *Uromyces phaseoli* inoculations than upper surface treatments against lower surface inoculations. With *C. lindemuthianum* inoculations, oligomycin gave good protection when applied to under surfaces whereas upper surface applications provided no protection.

374. Zentmeyer, George A., 1955, A laboratory method for testing soil fungicides, with *Phytophthora cinnamoni* as test organism. *Phytopathology* **45**, 398-404.

Of 47 chemicals tested for fungicidal activity in soil and *in vitro* using *Phytophthora cinnamoni* as a test organism, cycloheximide was one of 5 effective in agar and the only one active in soil.

375. Ziffer, Jack, Ishihara, S. J., Cairney, T. J. and Chow, A. W., 1957, Phytoactin and phytostreptin, two new antibiotics for plant disease control. *Phytopathology* **47**, 539. Abstract.

Laboratory and greenhouse tests were conducted with 2 polypeptide antibiotics, phytoactin and phytostreptin isolated from a *Streptomyces* species, against fungi and gram-positive bacteria. Low concentrations of both antibiotics gave effective control without host injury to early blight of tomato, late blight of tomato and bean rust.

376. Zukel, J. W., Smith, A. E., Stone, G. M. and Davies, M. E., 1956, Effect of some factors on the rate of absorption of maleic hydrazide. *Plant Physiology* **31**, suppl. XXI. Abstract.

The rate of absorption of maleic hydrazide (MH) by several plant species was determined by use of spectrographic analyses and isotope-tracer techniques. It was determined that the rate of foliar absorption of this organic compound (Mol. wt. 112) was directly proportional to the amount on the leaf surface. The greatest single factor affecting absorption rate was relative humidity. Temperature at controlled humidities had less of an effect upon the absorption rate.

CHAPTER V

ANTIBIOTICS IN FOOD PRESERVATION

BY C. L. WRENSHALL

A. INTRODUCTION

The preservation of food supplies has always been a vital concern of mankind—and still is today despite all the advances of modern technology.

Many of our methods of food preservation—such as drying, smoking, salting, fermentation, refrigeration, and even freezing—originated in prehistoric times. Modern technology has, of course, improved these methods almost beyond recognition and has extended their application to a greater variety of foods. Canning, based on the relatively recent discovery of heat sterilization and hermetic sealing, has become the most important general method of food preservation.

Despite the variety of methods available and their continuous improvement, many food preservation problems remain to challenge the food technologist. All the conventional methods have their limitations. They change flavor, consistency, nutritive value and other characteristics to a greater or less extent depending on the food product. Freezing provides perhaps the nearest approach to preservation in an unchanged state, but it is not always applicable and is relatively expensive. Simple refrigeration retards spoilage without significant change in flavor and structure, but is effective only for very limited periods of time.

Although it is difficult to cite definite figures, there is no doubt that throughout the world staggering losses of perishable foods occur due to spoilage. In America the annual spoilage bill runs into billions of dollars according to published estimates (U. S. Dept. Agr., Circ. No. 773, 1948; U. S. Dept. Agr., A.R.S. 20-21, 1954). Existing technology is apparently inadequate to cope with all the problems of food spoilage.

Through the insights of scientific investigation we have come to recognize that foods spoil through a number of independent mechanisms such as oxidation, enzymatic action and microbial growth. The last is, by far, the most important. The definition of a perishable food might well be that it provides an excellent medium for the growth of microorganisms. Of the three principal classes of food spoilage organisms—

molds, yeasts and bacteria—bacteria are the most destructive and dangerous.

Besides the economic benefits to be gained by preventing microbiological spoilage, there is room for great improvement in the quality of perishable foods. Some degree of deterioration of so-called "fresh foods" is now of necessity tolerated by the average consumer. Experience indicates that much of this loss of freshness—short of outright spoilage—is due to bacterial activity.

Food scientists are, therefore, still striving to find and develop better methods of controlling microbial spoilage. Both physical and chemical agencies—and various combinations of them—are being energetically explored. For example, extensive research is being conducted on the use of radiation to sterilize a variety of foodstuffs.

The search is also being continued for better antimicrobial compounds. The ideal compound would be nontoxic to man and higher animals but would, in very minute concentrations, inhibit the growth of spoilage organisms. It is thus only natural that the antibiotics, having proved so effective against pathogenic organisms in the fields of medicine, should receive consideration for their possible usefulness in food preservation. References to this possibility appear in the literature as early as 1945.

B. GENERAL CONSIDERATIONS

In relation to food preservation the antibiotics can be considered as a special group in the category of chemical preservatives. They differ from ordinary chemical preservatives in their biological origin and also in the degree of activity. The activity of the antibiotics is of a new order, one hundred to one thousand times greater than that of the conventional chemical preservatives.

In common with the chemical preservatives the antibiotics display considerable selectivity in their action. For example, penicillin is generally effective against the gram-positive bacteria whereas streptomycin is principally active against the gram-negative bacteria. The so-called broad-spectrum antibiotics, such as the tetracyclines, are inhibitory toward a much wider range of bacteria including both gram-negative and gram-positive types. These broad-spectrum antibiotics have proved to be of special interest in food preservation.

Although some early experiments indicated that chlortetracycline was more effective than oxytetracycline in inhibiting bacterial growth on foods, later investigations as well as numerous tests under commercial conditions showed that there is no practical difference between these antibiotics.

Relatively few of the well-known antibiotics display activity against fungi and yeasts and similarly most of them display little or no antiviral activity. The continuing search for new antibiotics may well, in the future, provide compounds active against these classes of organisms, but present antibiotic applications to food are directed primarily against bacterial spoilage.

Even where the broad-spectrum antibiotics are concerned it should not be inferred that all bacteria are susceptible to inhibition. It must be remembered that individual species and strains of organisms differ widely in their resistance to antibiotics. It is now recognized that, through selection or adaptation, strains can be obtained which show high resistance to individual antibiotics.

Another point that must be remembered is that broad-spectrum antibiotics at practical concentrations are not lethal even to the susceptible organisms. They merely inhibit growth and development. Thus, they do not sterilize but merely slow up bacterial spoilage and thereby prolong normal storage life. When microbiological deterioration does occur in antibiotic-treated foods, it proclaims itself by the common organoleptic signals of spoilage. Antibiotics cannot be used to cover up spoilage that has already occurred or to reclaim food that has become heavily contaminated.

Under natural conditions perishable foodstuffs have extremely short shelf-life, often measurable in terms of hours. This is because the bacteria on or in them proliferate rapidly, the total bacterial count doubling at frequent intervals, resulting in a "population explosion" (Fig. 5-1). The fresh life of the perishable food is the interval of time that elapses before the bacterial count goes into its steep climb.

Figure 5-1 shows the theoretical advantages to be gained by limiting the starting number of multipliers and slowing down the rate of doubling. *It is particularly significant that refrigeration and antibiotics supplement one another in this regard.* Generally speaking, the advantage to be gained by using antibiotics is greatly increased when refrigeration is also used.

Although total bacterial count is a commonly-used rough indicator of bacterial spoilage, it should not be inferred that there is a direct mathematical relationship between total count and spoilage as judged by organoleptic tests. Some of the types of bacteria present in a mixed flora are spoilage organisms, some are not, or at most contribute to spoilage in a minor way. This is particularly true of vegetable spoilage, where the so-called "soft rot" organisms are the chief culprits. One must beware of falling into the error of judging spoilage solely on the basis of total bacterial counts.

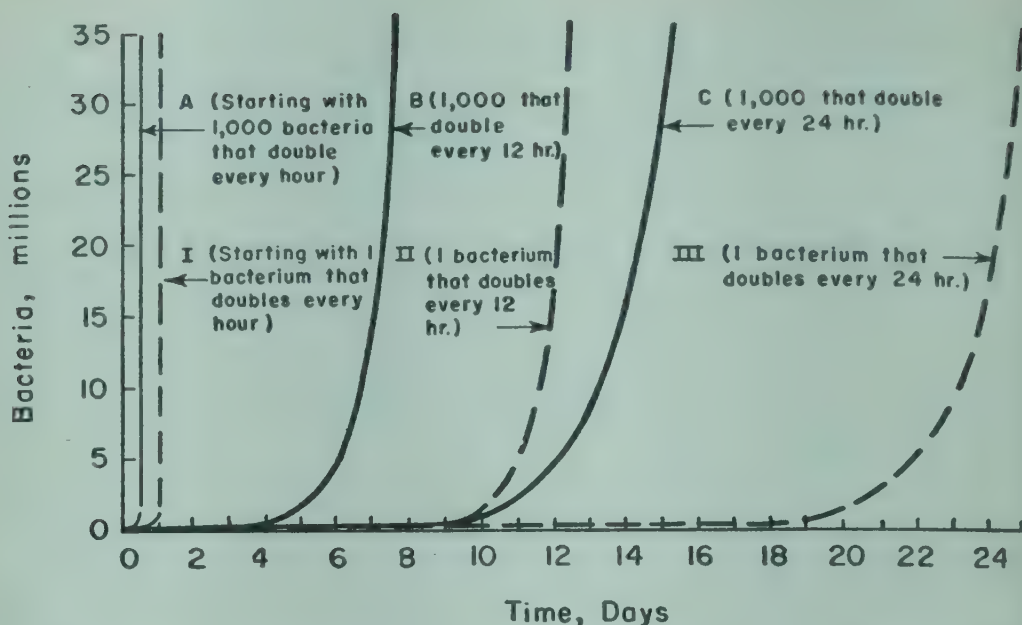


FIGURE 5-1. Bacterial population "explosions." BACTERIAL POPULATION "EXPLOSIONS" quickly limit a food's shelf-life, as depicted by these theoretical curves. Curve A traces population increase on starting with 1,000 bacteria that double every hour. Curves B and C show growth when bacteria double every 12 and 24 hours, respectively. Curves I, II and III give similar data with initial load of 1 bacterium. Spacing of these curves indicates the advantages of limiting the initial load of bacteria and slowing their rate of doubling. Food's shelf-life is the time interval before bacterial counts start to rise sharply.

Most of the antibiotics useful in foods are relatively labile substances which are readily inactivated by heat and which lose potency gradually on standing, especially in aqueous media. Generally they are relatively more stable at acid pH's and tend to lose activity rapidly in alkaline media. Thus, under the conditions of use in foods they may lose potency gradually during storage and are extensively destroyed at cooking temperatures.

These characteristics of antibiotics and their action make it clear that their application in food preservation is principally limited to use in conjunction with other methods. Used alone, they certainly do not afford a practical means of food sterilization. They can, however, slow down the rate of bacterial spoilage and used alone may have practical application in this regard. To some degree they can substitute for refrigeration. More important, their effect can be superimposed on that of refrigeration. Thus, there is considerable promise and, indeed, some present application of antibiotics in conjunction with conventional methods.

Considering all aspects, the following general applications for antibiotics in food preservation are contemplated:

1. As adjuncts to refrigeration in delaying the onset of spoilage of fresh perishable foods.
2. Delaying the deterioration of perishable foods during transportation and marketing.
3. To prevent the build-up of contaminants prior to other processing, e.g., in the preparation of fruits and vegetables for freezing.
4. As adjuncts in the sterilization of foods by heat or other means (e.g., radiation).
5. Preventing the growth of food poisoning organisms in processed foods.
6. Controlling contamination in biological processes such as fermentation.

C. ANTIBIOTICS IN CANNING

Canning is the most widely used method of food preservation. It is highly effective and applicable to most types of food. However, it is well recognized that it has certain defects, among which are (1) the loss of desirable flavor and texture through prolonged high-temperature processing; and (2) partial destruction of some of the vitamins, also due to processing at high temperatures.

In canning, antibiotics could assist in killing the resistant bacteria so that lower temperatures and shorter processing times would suffice to render the food commercially sterile. If they worked in this way, the antibiotics might help to improve the quality and raise the nutritive value of canned foods and perhaps decrease costs as well.

Perhaps not so widely recognized is the fact that commercially sterile does not mean completely sterile. As a result, economically significant spoilage occurs in most classes of canned products. Damage and loss due to "flat sours" and "hard swells" are almost universal, and are particularly heavy under adverse storage conditions. Thus, even if use of antibiotics failed to produce the advantages mentioned above, they might prove decidedly helpful if they merely reduced normal spoilage losses in canned commodities.

In May, 1950, Anderson and Michener reported the preservation of foods through the combined action of an antibiotic, subtilin, and mild heat. This announcement met with considerable acclaim and it appeared that antibiotics might have great significance for the canning industry. The propriety of adding to food an antibiotic substance such as subtilin was, of course, subject to question, but the preliminary indications were favorable and there was no *a priori* reason for ruling against it.

The first blow to the hope of combining canning methods with the use of subtilin came later in 1950 when Meyer of the University of California issued a public warning that the combination of subtilin and mild heat was not effective in killing the spores of *Clostridium botulinum*, deadliest of food-poisoning bacteria.

Morse discussed canning with antibiotics, pro and con. He questioned that subtilin is either a spore germination depressant or a sporicide, indicating that the antibiotic acts only on vegetative cells. For the proposed method to be 100% effective, there would have to be an effective residue of antibiotic throughout the shelf-life of the product.

Cameron, reviewing the situation for the American Meat Institute, reported that while subtilin and other antibiotics may reduce spore counts of *Clostridium botulinum*, *Bacillus thermoacidurans* and other spore-formers, the antibiotics cannot be relied on to produce complete sterilization in canned foods.

Cameron and Bohrer, presenting the viewpoints of the National Canners' Association, discussed the problem of proof of effectiveness of the antibiotics in canning. They emphasized that it would be necessary to have laboratory proof of the ability of the antibiotic to destroy spores of all organisms that must be controlled in practice. It is imperative to demonstrate beyond doubt that all spores of *Clostridium botulinum* will be destroyed.

Other papers have appeared dealing with various aspects of the antibiotic-mild heat problem without actually resolving it. In several instances subtilin plus 212°F for relatively short periods has sufficed to preserve uninoculated packs, but failed with inoculated packs, especially when inoculated with mesophilic bacteria, particularly putrefactive anaerobes, including *Clostridium botulinum*. In short, the method failed to pass the test of the National Canners' Association.

The fact that subtilin plus mild heat (212°F) failed to insure complete sterilization and safety under all conditions, together with the fact that subtilin has never become commercially available, caused most workers to lose interest in the application of antibiotics to canning. Emphasis shifted, both in academic and commercial circles, to study of the readily available tetracycline antibiotics in delaying the spoilage of perishable foods.

However, the subject of antibiotics in canning is not dead by any means. The studies of Stumbo and associates have demonstrated that the spores of *Clostridium botulinum* and Putrefactive Anaerobe 3679 are appreciably less resistant to heat in the presence of subtilin. Thus, it appears that practical conditions could be chosen under which the presence of subtilin could reduce the duration or intensity of processing

required in canning foods, or could make commercial sterility more nearly complete, thus cutting spoilage losses in commercial packs.

Rittenberg reviewed the subject of subtilin and mild heat to point out that the exact action of antibiotics on sporeformers is not known. Wheaton and his associates substantiated previous findings that flat sour spoilage of canned tomato juice can be controlled by addition of 5, 10 or 20 ppm subtilin combined with mild heat treatment. *Clostridium botulinum* is not a problem in this food.

In the early researches a number of antibiotics were tested, including chlortetracycline, bacitracin, chloramphenicol, gramicidin and others, but only subtilin showed any appreciable effect in supplementing heat in the destruction of sporeformers. However, a later report showed that oxytetracycline aided in the heat-destruction of several organisms with activity equal to or better than that of subtilin.

Recently the antibiotic nisin has been receiving much attention in Europe, especially in England. Nisin is very much like subtilin, both chemically and functionally, and shows great promise in canning applications. It is favorably regarded by public health authorities, since it sometimes occurs naturally in cultured dairy foods and it is not used in medicine. It is commercially available and its use is permitted in several countries. Hawley has recently reviewed its present and potential use in foods, including various canned and processed commodities.

Thus it is apparent that the last word has not been heard on the subject of antibiotics in canning. Further interesting developments in this area can be confidently anticipated.

D. FRESH FOODS

1. MEATS

As pointed out in the earlier discussion, the broad-spectrum antibiotics, particularly the tetracyclines, show considerable ability to inhibit the bacterial flora of perishable fresh foods. Of the various types of fresh foods none are of greater over-all importance than the red meats. Consequently a good deal of effort has been directed towards the application of these antibiotics to this type of food.

In general, two types of problems can be recognized—those having to do with the inhibition of surface contaminants, and those related to deep spoilage and the aging of carcasses.

Tarr and his associates were the first to report the effectiveness of tetracyclines in inhibiting the bacterial spoilage of fish and meat when applied to the surfaces by dipping or spraying, or by incorporation into minced tissue. Deatherage and his associates pioneered in the prevention

of deep spoilage and the tenderization of beef by infusion of the tissues with antibiotics. McMahan and associates developed the method of antemortem injection of oxytetracycline into cattle and other species.

In the latter method, the antibiotic is carried by the blood and lymph to even the most remote parts of the animal body. Moreover it affords the minimum opportunity for contamination of the deep tissues and maximum efficiency, since the inhibitory antibiotic is present before spoilage organisms can start to multiply. Various routes and sites of injection have been used satisfactorily, including intraperitoneal (cattle, sheep, hogs), intrathoracic (hogs), and intramuscular via the tail (cattle) and base of ear (sheep). Tissue levels of 1 ppm oxytetracycline or less have been found remarkably effective in delaying internal spoilage. Excellent steaks were cut from beef rounds hung over six weeks at temperatures varying from 40° to 80°F (average 55°F).

Comminuted meat products, such as hamburger, usually carry a heavy bacterial load and are highly susceptible to quality deterioration and outright spoilage. The fresh life of such products can be greatly extended by incorporation of antibiotics. Typically, adding 3 to 5 ppm of oxytetracycline to fresh pork sausage extends its fresh life by 50 to 100%. This treatment is surprisingly effective in maintaining fresh color.

Various cuts of meat, such as steaks, chops, roasts, can likewise be kept fresh much longer by dipping or spraying with dilute solutions of tetracycline antibiotics. The significance of this in the prepackaging of consumer cuts of fresh meats, possibly by the meat packer instead of at the retail level, is readily apparent.

Although only preliminary information is available, it appears that various processed items such as hams, bologna, frankfurters, pork sausages, dry salami, pickled tongue, etc. can also have their storage properties improved through the use of antibiotics. In certain products the antibiotic may be added to the curing pickle.

In the United States, animal carcasses are usually aged under refrigerated storage conditions in order to bring about, through the action of endogenous enzymes, desirable changes in flavor and tenderness. Traditionally, to combat spoilage, the carcasses are promptly chilled and stored under refrigeration. Unfortunately, low temperatures retard enzyme action; thus present methods are to a degree self-defeating. The introduction of tetracyclines, either by preslaughter injection or infusion, retards internal spoilage to such a degree as to permit rapid aging at relatively high temperatures, resulting in improved quality and reduced cost of storage. Deep spoilage or "sour," a serious problem with heavy beef especially when refrigeration is inadequate, is entirely prevented by such use of antibiotics. Indications have been obtained that lower-grade

carcasses can be improved through the more vigorous tenderizing action possible with antibiotics.

The antibiotic treatment of carcasses at slaughter is doubtless even more significant in areas where refrigeration is unavailable. In such circumstances problems of distribution are intensified and proper aging has hitherto been impractical. Under tropical conditions meat may become unfit for consumption within 24 hours of slaughter, hence must be consumed promptly. Excellent results were reported in early tests of antibiotic treatment by injection or infusion (with or without surface sprays) under such conditions. However, it remained for the recent studies of Ginsberg and associates in Kenya, and Matsui and associates in Japan to document and fully establish these methods. Most significantly, both groups found that surface sprays were highly effective in retarding spoilage and considered this to be the most practical way of applying the antibiotic.

In the Kenya experiments a significant number of animals (15 cattle, 13 sheep) were slaughtered under severe tropical and semitropical conditions and treated with oxtetracycline by various methods. As a practical matter, such treatments kept the carcasses fit for human consumption as judged by organoleptic tests for 48 hours in the case of sheep and up to 72 hours in the case of cattle. Extensive bacteriological tests confirmed the organoleptic observations and showed that potentially dangerous *Clostridia* and coagulase-positive staphylococci were effectively controlled. The spray method of application was recommended as a routine measure for the preservation of fresh meat in undeveloped areas.

In the Japanese study intensive bacteriological and organoleptic examinations were made on various tissues stored at 30°C, room temperature and under refrigeration. Differences between treated and untreated tissues were apparent at all temperatures, but were particularly significant in samples stored at high temperatures. *Clostridia* did not increase in antibiotic-treated samples regardless of storage temperature; this behavior was in marked contrast to that of the controls.

The effectiveness of the tetracycline antibiotics in retarding spoilage of flesh foods is rather difficult to explain solely on the basis of *in vitro* results. Jay and associates have noted that strains of organisms selected for antibiotic resistance are nonetheless inhibited by tetracyclines in the presence of beef tissue. Such inhibition can be reversed by adding various metallic ions to the meat; none of the organic additives tested was similarly effective. The hypothesis was advanced that the tetracyclines, which are chelating agents, compete with the contaminating organisms for the limited supply of metal ions in the substrate.

In recent years considerable work has been reported in the preservation of foods, especially meats, by ionizing radiations. A serious practical problem in this program has been the development of undesirable organoleptic properties when high radiation dosages are employed. This has led to investigation of the combined use of antibiotics and irradiation. The work of Niven and Chesbro showed there was considerable complementary effect of antibiotics and irradiation. Cain and associates found that radio-pasteurization dosages do not destroy the antibiotic activity and confirmed the pronounced complementary effects of oxytetracycline and irradiation. Presumably these results will lead to more practical procedures for radio-pasteurization.

2. POULTRY

In the United States the spoilage of chilled, unfrozen poultry has presented a serious distribution problem. Birds usually spoil in approximately one week at commercial storage temperatures. This spoilage entails great monetary losses. In addition, the consumer does not always obtain a first-quality product and must use the poultry within a day or two of its purchase.

Birds are normally chilled in a slush-ice tank after killing, plucking and evisceration. Adding 10 ppm of a broad-spectrum antibiotic to the slush-ice increases by 50 to 100% the time birds can be held without detectable spoilage.

Giblets usually are treated along with the birds in the chill tank. They are placed in a parchment wrapping which is then inserted into the bird's body cavity. Or they may be chilled separately in a 10-ppm antibiotic slush-ice mixture for 15 minutes, then packed with the birds as usual.

The U. S. Food and Drug Administration has established a tolerance of 7 ppm for residues of oxytetracycline or chlortetracycline in or on uncooked poultry. Such residues are unstable at cooking temperatures. Residues in poultry as eaten are undetectable.

However, poultry treated with an antibiotic must bear labeling stating that fact. An acceptable statement to comply is: "Oxytetracycline [Chlortetracycline] added to retard spoilage." The Food and Drug Directorate of Canada has also accepted this use of these antibiotics under similar regulations.

Antibiotic treatment has been applied mainly to chickens as broilers and fryers which make up the bulk of the poultry trade. The treatment has been particularly successful with whole birds but has also been applied with fair success to cut-up poultry. The latter presents special

problems and the extension of freshness that can be obtained is usually not as great as with whole birds. Undoubtedly, methods will have to be devised for antibiotic treatment of cut-up poultry. Antibiotic treatment has also been applied commercially to turkeys, ducks and other species.

Suppression of the bacterial flora gives rise to a different type of spoilage in which the predominant microorganisms are yeasts and molds. Considerable advantage is realized, however, as the yeasts and molds do not cause as rapid spoilage as the bacteria. Efforts are being made to suppress the yeasts and molds by the addition of antimycotic agents. The antibiotic, nystatin, has been reported to be effective in this regard.

Recently reports have appeared indicating that poultry processing plants have become contaminated with antibiotic-resistant spoilage organisms, with considerable loss of effectiveness of the antibiotic treatment. This has appeared to be a problem particularly in plants where the poultry is cut up and packaged. It is believed that the problem is principally one of plant sanitation to prevent build-up of large populations of resistant organisms on surfaces with which the poultry will come in contact during processing.

Considerable work is still going on to adapt antibiotic treatment to cut-up prepackaged poultry. The process of cutting up affords opportunities for contaminating the flesh with antibiotic-resistant bacterial flora as well as with yeasts and molds. However, it has been reported that vacuum packaging in nonpermeable films is advantageous in suppressing the yeasts and molds. Dipping or spraying the parts with stronger antibiotic solutions has also appeared helpful.

While the use of antibiotics in poultry processing has gained widespread acceptance in the United States and Canada, little interest has appeared elsewhere. This is attributable to the relative lack of refrigerated distribution in other countries. Stadelman and others have shown that antibiotic treatment affords little practical advantage unless the birds are effectively refrigerated at all times.

The British point of view is that there would be little practical advantage from antibiotic dips because of the general practice of distributing noneviscerated poultry, usually without refrigeration. They believe that under this system most of the spoilage organisms are invaders from the intestinal tract and it has been suggested that more advantage would be gained by having the birds ingest a quantity of antibiotic shortly before killing. It has also been pointed out that the practice of using the same antibiotics as poultry feed supplements may give rise to resistant spoilage bacteria.

In regard to the latter discussion, reports have appeared indicating that storage life of poultry may be extended by high level feedings of

chlortetracycline one to three days before killing or by giving the antibiotic in the drinking water for one day before killing.

It is apparent that the problems of delaying poultry spoilage have not all been solved by the simple dipping of carcasses in solutions of a tetracycline antibiotic. Considerable work is still going on to extend this approach, to combine it with other techniques, and to adapt it to distribution systems found in various parts of the world.

3 FISH AND SEA FOOD

Fish are probably the most perishable of all food commodities. The flesh of fish provides an ideal medium for the growth of bacteria. The economic and quality implications of spoilage are probably greater with fish than with any other class of food. It is significant that the ability of broad-spectrum antibiotics to retard bacterial food spoilage was first observed with fish. This discovery was the culmination of a long search by Tarr and his associates for chemical agents to improve the keeping qualities of fish.

Partly as a result of this early start, but also because of the world-wide importance of the problem, the use of antibiotics in fish and sea foods has received much study. A considerable body of published information is available, together with much practical experience, especially from Canada where the use of antibiotics in fish has been legalized for some time.

Many of the bacteria which cause fish spoilage are psychrophilic, or cold-loving, and therefore proliferate at refrigerated temperatures. Consequently, icing or refrigerated storage is only moderately effective in preventing spoilage. In general, the tetracycline antibiotics are most effective when used in conjunction with refrigeration. Some truly remarkable results in improving quality and extending normal storage life have been obtained through the combined effects of antibiotics and refrigeration.

Antibiotics may be applied to fish by spraying or dipping. These methods of application are particularly suitable where icing or refrigeration is not being used. Relatively concentrated antibiotic solutions are employed, i.e., spraying with a solution containing 100 to 200 ppm of antibiotic activity will approximately double the normal storage expectation. Dipping in solutions of 10 to 100 ppm will similarly extend the storage life.

When fish are iced on the boat, the antibiotic may be applied by spraying or dipping before icing. However, it has been found particularly advantageous to use ice which contains the antibiotic. The recommended

level of antibiotic in the ice is 5 ppm, although good results have been reported using even lower concentrations.

When flake ice is manufactured, the required level of antibiotic is simply dissolved in the water before freezing. However, when ice is frozen in blocks, dissolved materials including the antibiotic tend to concentrate in a small core near the center of the block. This tendency can be largely prevented by dissolving hydrophilic colloids, such as carboxymethylcellulose or carrageens, in the water or brine before freezing. Practical formulations of these colloids with the antibiotics are offered commercially for the manufacture of antibiotic block ice.

On some modern fishing boats the catch is stored in refrigerated brine. In this method the antibiotic is simply dissolved in the brine. This method of storage is particularly efficient and antibiotic levels as low as 1 ppm are effective.

It should be pointed out that the antibiotics are more effective in eviscerated fish than in whole fish since in the latter they do not inhibit visceral bacteria. However, the slime bacteria on the surface are of greater importance in fish spoilage and considerable extension of freshness is obtained by the antibiotic treatment of whole fish. As examples, whole red fish remained fresh for 20 days under antibiotic ice, while the controls spoiled in 14 days under ordinary ice; eviscerated haddock remained fresh for 25 days under antibiotic ice, while the controls spoiled in 13 days.

The dipping of fillets in antibiotic solutions has proved to be remarkably effective in extending their freshness. Fish fillets are usually dipped in a brine solution just before packaging. If a tetracycline antibiotic is added to the brine at levels of 10 to 100 ppm, freshness may be extended as much as 300%. For example, in a commercial experiment with oxytetracycline, haddock fillets were dipped for 30 seconds in the following brine solutions: untreated, 10 ppm, 25 ppm and 50 ppm. Stored at 0°C, the untreated fillets lasted seven days, the 10-ppm treated lasted 14 days, the 25-ppm treated lasted 20 days, while the 50-ppm treated fillets remained in good condition for more than four weeks.

Actually, the dipping of fish fillets is the principal commercial use of antibiotics that has developed to date in the fishing industry in Canada. This is probably because the United States authorities have not yet approved the application of antibiotics to fish and a large part of the Canadian catch is sold in the United States. It may be anticipated that the use of antibiotic ice on fishing boats will become prevalent after United States approval of use of antibiotics in fish. It would obviously be of great value to apply the antibiotics as soon as possible after the fish are caught.

Generally speaking, the experimental application of antibiotics to shellfish has not been as successful as was expected from the results with fish. There is no ready explanation of this difference. The results obtained with shrimp have been disappointing. In some experiments with crab and oyster meats the bacterial loads were reduced but shelf-life was not extended. However, in other tests chlortetracycline showed promise in maintaining the freshness of shucked oysters. In an experiment with shucked clams, dipping in a 5-ppm oxytetracycline solution extended the storage life 28 days at 0°C. Also, excellent results were obtained by dipping cooked lobster meat in oxytetracycline solutions.

Large quantities of fish, notably menhaden, are processed for the production of fish oil and fish meal. Bacterial decomposition is an extremely serious problem in such fish and losses may amount to 20% or more, being especially heavy in warm weather. Although the economic margins are narrow, broad-spectrum antibiotics can be used to retard deterioration. Useful results have also been obtained in handling trash fish which are processed for animal feed and prepared foods for domestic animals.

Whales present a unique spoilage problem because they are warm-blooded mammals and also because of their great bulk and the insulating effect of the blubber. Moreover, the whale's visceral tract is heavily loaded with anaerobes such as those of the *Clostridium* genus. Extensive bacterial action begins almost immediately after killing and is manifested by elevated temperatures and putrefactive changes within 24 hours. Unless processed very soon after killing, not only is the meat spoiled for edible purposes but the quality and yield of oil and meal are greatly reduced. The introduction of antibiotics at the time of killing is of great value in delaying spoilage. Antibiotics were introduced by placing them in the exploding head of the harpoon, but it was found that this method is rather unreliable. More consistent results have been obtained by pumping antibiotic solutions into the abdominal and chest cavities through the airline used to inflate the whales. The cost of the antibiotic—about 50 grams per whale—is insignificant in comparison with the economic benefits. Consequently, the use of antibiotics has been rapidly adopted by the whaling industry.

It has been shown repeatedly that only minor concentrations of tetracyclines are retained by the fish tissues when treated in the various ways suggested. Dipping briefly or spraying, even with relatively high concentrations, produces negligible antibiotic residues. Longer treatments, such as storage in antibiotic brine or antibiotic ice, may introduce appreciable levels of antibiotic into the flesh. Such residues, however, are reduced to insignificant levels by cooking.

4. FRUITS AND VEGETABLES

The extremely perishable nature of fresh vegetables, especially the green leafy varieties, is one of the most adverse factors encountered in their distribution. Substantial losses due to spoilage are accepted as normal and are a major factor in determining the price consumers must pay for such products. The need for methods of delaying vegetable spoilage has been accentuated by recent trends in distribution involving the pre-packaging of these commodities in ready-to-use form. The most prevalent cause of spoilage is bacterial soft rot.

Modern methods of handling raw agricultural commodities after harvest materially reduce development of bacterial spoilage. Such methods as hydrocooling and refrigeration, however, only inhibit microbial decay by the influence of temperatures adverse to growth of the microorganisms. When perishable produce is removed from these conditions, spoilage often occurs quite rapidly.

The need for better control of bacterial soft rot in vegetables has created interest in the possible use of antibiotic treatments. Bonde observed control of soft rot of potatoes by dip treatments of streptomycin and oxytetracycline. Smith reported the reduction of bacterial soft rot of prepackaged spinach with treatments of streptomycin sulfate and oxytetracycline. Cox demonstrated the effectiveness of streptomycin and combinations of streptomycin sulfate with oxytetracycline as post-harvest treatments for prevention of bacterial rots of lettuce. Brody and Francis reported 40% reduction of soft rot decay in prepackaged spinach by a one-minute dip in a 500-ppm solution of streptomycin sulfate. Koch and Carroll reported reduction in decay of spinach with oxytetracycline, streptomycin, polymyxin and neomycin dip treatments. Cox and associates also reported complete control of radish pit after five days' storage at temperatures of 5° to 35°C, using five-minute dip treatments of 40-ppm oxytetracycline solutions. Streptomycin at the same concentration was not as effective and did not give control at 35°C.

Carroll and associates studied decay in a salad mix and each of its components, using dip treatments of antibiotics. Vegetables which deteriorate rapidly appeared to establish the rate of decay for the other components in a salad mix. Degree of spoilage could be judged easily by measuring the volume of liquid exudate. The effects of antibiotics and refrigeration were additive. Oxytetracycline was by far the most effective antibiotic tested.

Goodman and associates confirmed the effects of antibiotics in prolonging the refrigerated shelf-life of spinach. They also studied the active antibiotic residues present in various vegetables after treatments

with oxytetracycline and streptomycin. Residues of streptomycin were found to be persistent and there was no assurance that they would be destroyed by cooking.

Fungi are responsible for a considerable amount of postharvest spoilage, especially of fruit products. Typical examples are blue mold of citrus and brown rot of peaches. It is possible that antifungal antibiotics will be developed which will be useful in controlling such spoilage. Nystatin has shown considerable effectiveness in this regard, as reported by DiMarco and Davis.

While the information available is still somewhat fragmentary, the future appears to hold considerable promise for the use of antibiotics on vegetable products. Certainly, antibiotics appear capable of contributing to the solution of serious spoilage problems in this area. Realization of these potential values must, however, await clarification of the public health significance of antibiotic residues.

5. DAIRY PRODUCTS

In technologically advanced areas there is little reason to consider the possible use of antibiotics to keep milk fresh. However, fresh milk is unavailable to much of the world's population, partly because of the impossibility of distributing it without adequate pasteurization, refrigeration and transportation facilities. Consequently, some study has been given to the possible use of antibiotics in this regard.

Early observations indicated that penicillin, streptomycin and other narrow-spectrum antibiotics, while capable of interfering with lactic acid production (souring), were of little value against putrefaction. Various combinations were somewhat more effective, but as observed with other foods, the broad-spectrum antibiotics proved to be of the greatest value. Several reports indicate that chlortetracycline or oxytetracycline can substitute to a considerable extent for refrigeration. If 1 ppm of a tetracycline antibiotic is added to raw milk directly after milking, the onset of spoilage is delayed for about one day at 37°C. If the milk is pasteurized, these antibiotics will preserve it without refrigeration from two days to several weeks, depending on storage conditions and the level of antibiotic used.

Some attention has been given to the use of antibiotics in the preservation of human milk. Chlortetracycline was reported more effective than streptomycin, which in turn was more effective and better tolerated than citric acid.

One other aspect of antibiotics in market milk deserves mention, although not related to food preservation. That is the incidental

contamination caused by treatment of mastitis with antibiotics. Penicillin is the antibiotic contaminant most commonly encountered, although other antibiotics used to treat mastitis are detected occasionally.

Contamination of milk with antibiotics is objectionable from two standpoints. They interfere with acid production and hence with the manufacture of cheese and other cultured dairy foods; they are objectionable from a public health standpoint and milk containing them is considered adulterated. Penicillin has been of particular concern because of the possibility that it might sensitize or cause allergic reactions in persons already sensitized.

Low-level antibiotic residues are remarkably stable in milk. Temperatures encountered in pasteurization are relatively ineffective in destroying them. There is evidence that some constituents of milk exert considerable protective influence.

The antibiotic nisin, produced by various strains of the cheese-starter organism *Streptococcus lactis*, and therefore present naturally in some cheeses, can be used to very good advantage in cheese making, especially in the manufacture of processed cheese products. It is a narrow-spectrum antibiotic, quite similar in its properties to subtilin. Being inhibitory to Clostridia and certain other spoilage bacteria, it may well have applications in other fields of food preservation, as noted elsewhere.

The conditions in processed (pasteurized) cheese products are often ideal for the development of Clostridia, leading to spoilage of the cheese, frequently with gas formation. Several of the Clostridia found in processed cheese are capable of producing toxins, hence are objectionable from a public health standpoint. It has been reported that these problems can be solved by the use of nisin, which is now available commercially. Use of nisin is permitted in Britain and several European countries.

Clostridia may also cause serious defects in various original cheeses, including many of the Continental types. The use of nisin-producing cultures and of commercial nisin to prevent these defects is being studied with promising results, but has not attained a commercial status.

Preliminary results have been reported (Elliott and Romoser) on the prevention of green-rot spoilage of eggs by means of antibiotic dips. It was observed that egg albumen interferes with the antibiotic assay, but by suitable dilution antibiotic activity was detected in various components of the egg after soaking 10 minutes in chlortetracycline solutions. Further work is desirable, as bacterial spoilage causes serious losses in market eggs.

6. YEAST FERMENTATION

Most of the well-known antibiotics are active against bacteria but have little or no effect against yeasts. This situation has led to study of their use in commercial fermentation and yeast production to inhibit the development of bacterial contaminants. While several different antibiotics have proved useful in this regard, usually one proves to be most effective and economical in a specific situation. For example, Day and associates reported that penicillin excelled the broad-spectrum antibiotics in suppressing bacterial development in grain alcohol fermentation.

Strandskov and Bockelmann found it possible to control bacterial contamination encountered in brewing by incorporating the antibiotic polymyxin B in the yeast inoculum. As little as 0.005 unit per ml of polymyxin in the fermenting beer eliminated the growth of gram-negative contaminants. Fermentation was actually stimulated and there was also the possibility of substantial saving by keeping the yeast relatively free from contamination and suitable for re-use. Polymyxin activity was entirely lacking in the finished beer.

In the production of yeast, bacterial contamination may considerably lower yields. Inoculum is prepared in small volumes grown under aseptic conditions, but production takes place in open tanks with capacity up to 25,000 gallons that are freely accessible to air-borne contamination of primarily gram-positive organisms. Commercial experiments have shown that penicillin at a concentration of as little as 5 units per ml controls the contamination. Greater yields of yeast are obtained, owing to suppression of the bacteria which would compete for nutrients. Some studies also show that the rate of growth of the yeast may be stimulated by this method. The normal washing of the yeast removes any antibiotic which may be present. It also has been observed that yeast cakes produced in this manner have a longer shelf-life.

Several reports have appeared discussing the possible application of antibiotics in wine making. However, so far as is known, there is little or no actual use of antibiotics by the wine industry.

E. PROCESSED FOOD POSSIBILITIES

There seems to be little chance that antibiotics will supplant any of the conventional methods of food preservation. Judging by the literature already reviewed, the most important use of antibiotics in foods is to delay the spoilage of perishables. Such use of antibiotics may well supplement preservation by conventional methods like freezing, canning, dehydration or curing. An example of this is found in the work of Stern and associates who observed significant improvement in canned salmon

when antibiotic was used to inhibit bacterial deterioration occurring prior to canning. It has also been suggested that antibiotic dips afford a means to keep bacterial counts of frozen foods within acceptable limits.

Hawley has suggested that nisin may be used, not only to improve commercial sterility in canned packs (as discussed earlier), but also to improve various nonsterile foods. For example, he proposes that nisin can prevent defects in cured or pasteurized meat products, like the "greening" caused by lactobacilli or the "bone-taint" of hams caused by lactic acid bacteria. He also suggested that nisin could be useful to combat various bacteriological defects in canned hams, open-pack meats and sausages, pickles, chocolate milk, cream for butter, and gelatin. However, most of these suggestions have not yet been supported with published data.

A preliminary study by Ordal and associates indicates the potential of the tetracyclines in supplementing the curing of hams and the possibility of developing milder cures. A recent report from Finland (Uusimaki) describes an "experiment" in which oxtetracycline was used in processing over a million kilograms of cucumber pickles with excellent results. The damage caused by microorganisms was reduced to $\frac{1}{10}$ of that shown in the previous year's trials.

The continuing trend to convenience foods—prepared mixes, ready-to-cook and ready-to-eat items, for instance—poses many novel variations of food preservation problems. Godkin and Cathcart recognized the potentialities of antibiotics in the spoilage of prepared custards. They showed that a combination of oxytetracycline and subtilin prevented the growth of both food-poisoning organisms and spoilage organisms for three days at summer temperatures. Other than their reports, the published literature offers little. It is known that consideration is being given to the possible use of antibiotics in prepared biscuit doughs, precooked frozen meals (meat pies, etc.), ready-to-cook pancake mixes, breading batters, and frozen soup mixes, to name a few obvious possibilities. Almost every food technologist recognizes a possible application of antibiotics in some specific problem with which he is familiar. All such uses must be thoroughly investigated before any conclusions can be arrived at, as antibiotics are certainly no "cure-all" for problems in food bacteriology and sanitation.

F. ANTIBIOTIC RESIDUES

Most food regulatory agencies are understandably cautious in their attitude toward the addition of antibiotic substances to food. So used, the antibiotics fall into the class of chemical food additives and it is only fitting that they should be subject to the appropriate criteria for

safety in use. With antibiotics there is the additional consideration that their value as drugs is of paramount importance and must be safeguarded. Particular concern has been expressed over the possibility that active residues of antibiotics might: 1) sensitize consumers or cause allergic reactions in those already sensitized; 2) lead to the emergence of resistant pathogens which would not be controlled by therapeutic doses of antibiotics.*

Because of this concern a great deal of painstaking effort has been directed towards the detection and measurement of antibiotic residues. The task has been complicated in some instances by the presence of natural antibiotic activity in the foods, in others by the presence of interfering substances. In most cases the attempt has been made to measure residues below the sensitivity of the assay method by extracting the antibiotic and concentrating it. Since most antibiotics are rather unstable and are rapidly destroyed at cooking temperatures, particular attention has been given to the active residues persisting in the foods as finally eaten.

In the case of poultry it has been amply demonstrated that the antibiotic activity introduced into the tissues by commercial treatment with chlortetracycline and oxytetracycline is reduced below detectable levels by normal cooking. The residue tolerance of 7 ppm maximum in any portion of raw poultry, permitted by United States and Canadian agencies, is in effect a zero tolerance in the food as eaten.

Canada has permitted oxytetracycline and chlortetracycline treatment of fish, provided the antibiotic activity in the raw flesh does not exceed 5 ppm. Practical treatments have been found to produce concentrations up to this level only in the skin and visceral cavity wall and then only after prolonged soaking. Brief treatments, such as the dipping of fillets or the spraying of whole fish, produce barely detectable levels. Normal cooking of fish, which may not be as thorough as that of poultry, normally destroys all the residual antibiotic activity. In a few instances slight residues have been reported which presumably are not regarded as significant by the Canadian authorities. However, United States authorities have taken the attitude that it is necessary to prove the harmlessness of these residues.

In meat preservation antibiotic levels of about 1 to 2 ppm in the muscular tissues are introduced by injection or infusion with tetracyclines. The liver and kidneys take up much more injected antibiotic than the other tissues. Since times and temperatures involved in cooking meat (especially beef) are highly variable, complete destruction by cooking

* For a fuller discussion of the public health aspects of this subject see Chapter VII.

cannot be assured. However, it is noteworthy that sprays or dips produce slight antibiotic residues in the surface layers only, and these residues may be undetectable after 24 hours, as shown in the Kenya experiments.

The available evidence indicates that there is little or no absorption of antibiotic by various vegetable tissues subjected to dip or spray treatments with tetracyclines. Residues detected probably represent mostly antibiotic dissolved in surface water, since rinsing removes most of it. However, the possible public health significance of residues on vegetable products requires further study.

One further aspect of residues from tetracycline antibiotics requires comment. Questions have been raised about the toxicology of the heat degradation products. Shirk and associates reported that the principal heat degradation product of chlortetracycline in poultry flesh is isochlortetracycline which appeared to be nontoxic. The heat degradation products of oxytetracycline have been found to be innocuous. Indirect evidence from the long-term feeding of these antibiotics makes it seem very unlikely that there is anything to fear from their break-down products.

It has been reported that limited use in foods of tetracycline antibiotics has been permitted in the following countries: Brazil, Canada, Chile, Colombia, Costa Rica, Cuba, Finland, Greece, Guatemala, Iran, Japan, Mexico, Panama, Philippines, Spain, United States.

The status of nisin is somewhat different from that of the tetracyclines in that it is not used as a drug, it is present at times in some natural foods, and it is destroyed by gastric digestion, hence it is unlikely to affect the intestinal flora. Available evidence indicates that it is nontoxic. Use of nisin, at least in cheese, appears to have gained approval in England and various European countries and favorable consideration in Australia.

G. ECONOMIC AND OTHER BENEFITS

The preceding sections have outlined the various documented uses of antibiotics in food preservation. The fact that so much work has been reported is itself a testimony to the existence of unsolved technical problems. However, when new food additives are proposed for use, evidence should be presented to show that benefits to the consumer will result. It is therefore pertinent to review the available evidence on this point.

It is self-evident that, since no segment of the food industry plans to operate at a loss, the cost of all spoilage is ultimately borne by the consumer. The price he pays includes a factor to cover spoilage which

occurred en route to the counter; spoilage that occurs thereafter is his liability.

Unfortunately it is difficult to put a price tag on losses due to bacterial spoilage—and next to impossible, at this stage, to estimate the savings that may finally result from the use of antibiotics. However, available statistics and estimates all indicate that such savings may be very large. For example, the U.S.D.A. estimate of marketing losses in poultry in 1954 was \$268 million, or 9.7% of the total value of the poultry products marketed. The poultry losses may be taken as a rough approximation of the losses to be expected in marketing livestock products. The total value of livestock products was almost \$16 billion in 1954. These comparisons give some impression of the large savings which may be realized by reducing spoilage losses.

In vegetable products, percentage losses run much higher—as high as 43% of some perishable fruits and vegetables. Estimates of United States losses of fruits and vegetables run to over \$1 billion a year. Bacterial spoilage accounts for much of this loss. In a study of spoilage in carload shipments of vegetables, bacterial soft rot was by far the chief cause of spoilage. In this study, of about 100,000 carloads of vegetables shipped from Florida to New York (1952–53) the equivalent of 4,000 carloads was discarded on receipt for a total spoilage loss of nearly \$5 million. There is no doubt that such losses occur in all the channels of distribution, reaching their peak in the retail store and the consumer's refrigerator.

The fishing industry provides perhaps the most striking examples of what may be accomplished through the use of antibiotics. At the dock fish are cheap; for example, Canada catches 2 billion pounds worth about 5 cents a pound. But at inland points, fresh fish are expensive, costing many times this price if available at all. Yet an inexpensive antibiotic dip makes it possible to distribute fresh fish fillets from coast to coast.

From the standpoint of the fishing industry the potential value of antibiotics is attractive. Boats may be enabled to stay out longer and range farther, making a profitable catch more certain—and incidentally helping to ensure a steady supply of fish to the market at reasonable cost.

The cost of antibiotic treatment is small. Treatment of poultry as currently practiced in the United States and Canada is less than $\frac{1}{2}$ cent per pound. The cost of antibiotic for packing fish in antibiotic ice and the cost of dipping fish fillets in antibiotic solutions is of the order of $\frac{1}{10}$ cent per pound. Dips for vegetable produce will cost about the same in general. The proposed preslaughter injection of antibiotics will cost at most $\frac{1}{2}$ cent per pound of meat. It is believed that these costs are trivial in relation to the benefits to be obtained from the treatments.

The Joint FAO/WHO Expert Committee on Food Additives has

pointed out that high temperatures and humidities encountered in the tropics favor deterioration and justify greater use of additives than in more temperate climates. It is true that because of these conditions, coupled with the lack of sanitation and refrigeration, meat must be distributed and eaten within 24 hours, fish are unavailable as little as a few miles inland and such milk supplies as are available cannot be distributed in the tropics. The results already cited indicate the utility of antibiotics in the distribution of these valuable foods. Besides opening up new economic frontiers, the ready availability of these foods may help to overcome serious nutritional deficiencies. In particular, the protein deficiency disease kwashiorkor could probably be overcome with adequate local supplies of milk and other high-quality proteins.

A benefit to consumers that is difficult to evaluate in economic terms is the quality improvement that results from the early application of antibiotics. This is particularly true of fish and sea food—almost everyone has expressed how good they can be when they are truly fresh. It is also valid in relation to most other perishable foods. Much of this loss of freshness, short of outright spoilage, is due to bacterial activity and can be prevented by the appropriate use of antibiotics.

A great deal of research is now being directed toward the tenderizing of beef. This problem is growing more important, especially in the United States where the public constantly demands larger supplies of tender beef. It is believed that adequate tenderization could add 3 cents a pound to the value of beef at retail. The high temperature aging made possible by antibiotic injection aids materially in the tenderization of beef carcasses.

By the use of antibiotics in conjunction with rapid transportation (e.g., air freight) a greater variety of fresh food delicacies may become possible, with consequent economies. For example, retail cuts of fresh meat, cut-up poultry and fish fillets may be consumer packaged at the processing plant.

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Anon., 1954, For antibiotics, uses galore. *Chem. Eng. News* **32**, 4640.

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- ✓ Ingram, M., Barnes, E. M. and Shewan, J. M., 1956, Problems in the use of antibiotics for preserving meat and fish. *Food Sci. Abstr.* **28**, 121.

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Problems of preserving meat, vegetables and fish are examined with particular attention to the better utilization of raw materials and the conventional applications of high and low temperatures. Emphasis is laid on the new techniques of using antibiotics and ionizing radiations; some aspects of the problems arising are considered. Progress in the manufacture and use of containers is mentioned, and some future trends briefly discussed.

Deatherage, F. E., 1957, Use of antibiotics in the preservation of meats and other food products. *Am. J. Public Health* **47**, 594.

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Esselen, W. B., 1957, Food preservation and its contribution to nutrition. *Borden's Rev. Nutrition Research* **18**, 29.

A short review is given of the use of antibiotics. Immediate applications of antibiotics appear to be as agents to retard spoilage in refrigerated fresh products such as prepackaged vegetables, fish, meat and poultry. If and when satisfactory uses of antibiotics are developed for reducing the heat process requirements of canned foods, the resulting products should exhibit a better retention of nutrients and fresh quality characteristics.

Jukes, T. H., 1957, Antibiotics, nonmedical uses, in Kirk, R. E. and Othmer, D. F., Editors, *Encyclopedia of Chemical Technology*, First Supplement Volume, Interscience Encyclopedia, Inc., New York, 59.

Use of antibiotics in animal and poultry feeds, in the treatment of plant diseases and in the preservation of food is reviewed. The possible sources of antibiotic residues in food for human consumption also are considered.

Reith, J. F. and Mossel, D. A. A., 1957, Symposium on preservation of foods. 10. Preservation by use of chemical compounds (in Dutch). *Conserva* 5, 328, 353.

All chemical compounds commonly used in the food industry are considered in these review articles. Other food preservatives are acidic and their action is a function of pH. Antibiotics are specific against certain organisms. The authors concluded that more experiments are necessary to determine the possible toxicological effect in humans of the use of antibiotics.

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Hygienic conditions in the handling of meat and fish products decrease the bacterial contamination and, with refrigeration, are a great aid to maintaining freshness. Antibiotics can extend the freshness of meat and fish and many have been tested experimentally for their value. The broad-spectrum antibiotic, chlortetracycline, is in use in the United States, Guatemala, Colombia and Iran for the treatment of poultry carcasses. Before antibiotics are generally used in foods the possible induction of bacterial resistance and the occurrence of antibiotic sensitivity must be considered. An outstanding application of their use would be in meat distribution in tropical climates so that large communities would, for the first time, receive a continuous supply of fresh animal protein.

Thieulin, G., 1957, Better methods for preservation of agricultural products and fish (in French). *Compt. rend. acad. agr. France* 43, 336.

The beneficial aspects of the use of antibiotics in certain cases of food preservation, especially of fish, meat and poultry, the effects of ionizing radiations on foods and the total effects of these methods in comparison with those of long established methods of preservation by heat or cold are discussed.

Taylor, J. H. and Walker, E. A., 1958, Recent developments in food preservation technology. *Research, London* 11, 61.

Recent developments in food technology are outlined. Various ancient methods of food preservation are in use but advances have been made in preserving the flavor, odor and freshness of foods. Existing packaging and freezing methods have been improved and progress may be extended by use of ionizing radiations and antibiotics.

C. ANTIBIOTICS IN CANNING

Anderson, A. A. and Michener, H. D., 1950, Preservation of foods with antibiotics. I. The complementary action of subtilin and mild heat. *Food Technol.* 4, 188.

Mild heat is necessary in treatment of vegetables to inactivate enzymes which would otherwise cause deterioration. The heat is sufficient to destroy yeasts, fungi and all non-sporeforming bacteria, some of which are resistant to subtilin.

Clostridia and thermophiles are resistant to heat but are extremely sensitive to subtilin. The following facts suggest that subtilin would be harmless even with continued use:

- ✓ 1. Subtilin is a polypeptide and is inactivated (presumably hydrolyzed) by proteolytic enzymes.
2. 100 ppm in rat diets has not affected growth or health over a period of 175 days.
3. Oral dose (1 gm per kg) to a rabbit caused no ill effects.
4. *Bacillus subtilis* often is found in foods where it may have produced appreciable quantities of subtilin, but no case of food poisoning is attributed to it.
5. The microflora of intestinal tract presumably is not affected because of no antibacterial effect against gram-negative bacteria.

Morse, R. E., 1950, Canning with antibiotics—pro and con. *Food Inds.* **22**, 1679.

Five to 20 ppm subtilin and mild heat, consisting of boiling water for about 10 minutes, successfully preserves canned vegetables. Inoculated with *Bacillus stearothermophilus* and *Clostridium botulinum*, packs remained unspoiled for 8 months.

✓ Advantages of antibiotic preservation canning:

1. A method which could be adapted to automatic processing at a moderate cost.
2. Eliminate batch processing (high labor cost).
3. Reduce processing time (long time, high-temperature).
4. Raise the productive capacity especially where the processing unit is the limiting factor.
5. Higher quality products due to short-time, low temperature (higher nutritional value).
6. Benefit to marginal income group of canners.

✓ Disadvantages:

1. Must be 100% effective and demonstrate safety factor (not shown yet with antibiotic-mild heat).
2. Ability of organism to undergo selective adaptation.
3. Antibiotics which have medical uses will have limited use in canning because the consumer might become insensitive.
4. Continued ingestion could possibly bring a change in intestinal flora (subtilin is a simple polypeptide and thus presumably disposed of early in the intestine).
5. Heat is to eliminate yeast and molds while antibiotic is to eliminate the more resistant spores, but studies have indicated the antibiotic was neither a spore depressant nor a sporicide but organisms were eliminated at the time of germination. For the method to be effective there must be a residue throughout shelf-life of the product.
6. Problem of mutation.

Adams, A. T., Ayres, J. C. and Tischer, R. G., 1951, The effect of subtilin and heat on inoculated beef in tubes. *Bacteriol. Proc.* **51**, 16.

See also Adams, A. T., Ayres, J. C. and Tischer, R. G., 1951, Effect of subtilin and heat in preventing spoilage of comminuted beef. *Food Technol.* **5**, 82.

Comminuted beef was inoculated with Putrefactive Anaerobe 3679 (100, 10,000 and 1,000,000 spores per gram) and treated with 10, 100 and 1000 ppm subtilin and mild heat. Higher levels extended the time before spoilage. The antibiotic is believed to inhibit spore germination and not to destroy spores.

Bohrer, C. W., 1951, N. C. A. experimental program with subtilin. Nat. Canners' Assoc. Information Letter No. 1325, 21; Also, Denny, C. B., Bohrer, C. W. and Cameron, E. J., 1951, Nat. Canners' Assoc. experimental program with antibiotics. Research Report, No. 7-51.

In the experimental program with subtilin, the basic requirement of the antibiotic would be to prevent *Clostridium botulinum* toxin growth. If the antibiotic were completely effective against botulism, the secondary interest would be against other important spoilage organisms.

The sensitivity of the principal spoilage organisms to subtilin and mild heat was tested. Thermophilic types are more sensitive to subtilin than mesophilic types. Within the thermophilic group, the flat sour types appear most sensitive, while in the mesophilic group, the putrefactive anaerobic types, which include *Cl. botulinum*, are the least sensitive. The effect of the subtilin doesn't persist over an extended incubation period. Growth usually occurs in tubes containing 10 ppm of subtilin after 60 days incubation. When no deliberate inoculation was made, 20 ppm subtilin appeared sufficient to prevent spoilage in all products except broccoli and celery. The general indications are that subtilin loses its potency with storage and the rate of this loss appears to be influenced by the nature of the food product to which it is added and by the storage temperature.

Burroughs, J. D. and Wheaton, I. E., 1951, The preservative action of antibiotics in processed foods. *Canner* 112 (10), 50.

Subtilin, gramicidin, methylolgramicidin, bacitracin and streptomycin were added to experimental packs of peas and corn in brine, and the packs were inoculated with a spore suspension of *Clostridium botulinum* containing 5 strains each of type A and type B. The packs were processed at 212°F. A subtilin concentration as high as 80 ppm permitted 100% spoilage. Similar results were obtained with the other antibiotics tested in a single high concentration. None except subtilin even controlled the natural bacterial flora of the vegetables. Spores of *Bacillus thermoacidurans* were used for an experimental pack of tomato juice processed with subtilin and mild heat treatment. No evidence of spoilage was found with 40 ppm of subtilin.

Cameron, E. J., 1951, Use of antibiotics in preserving foods. *Proc. Conf. on Research, Council on Research, Am. Meat Inst., Univ. Chicago, 3rd Conf.*, 32.

While subtilin, gramicidin, methylolgramicidin, bacitracin and streptomycin reduce spore counts on *Clostridium botulinum*, *Bacillus thermoacidurans* and other sporeformers in canned foods, they cannot be relied on as aids to canned food sterilization.

✓ Cameron, E. J. and Bohrer, C. W., 1951, Food preservation with antibiotics: The problem of proof. *Food Technol.* 5, 340.

✓ The elements of proof that will be necessary to establish the acceptability of any antibiotic in food preservation are: 1, the antibiotic must destroy spores of *Clostridium botulinum*; 2, it must destroy spores of saprophytic organisms;

3, it must be nontoxic and otherwise meet public health requirements; 4, it must meet food law requirements; and 5, it must be economically practicable.

Magdonelle, F. L., 1951, Research on the antibiotic activity of subtilin (in French). *Compt. rend. soc. biol.* **145**, 1876.

Since the spectrum of subtilin is selective, the material to be preserved should be partially sterilized before its addition.

✓ Oleott, H. S., 1951, Problems in the application of antibiotics to food processing. Nat. Canners' Assoc. Information Letter, No. 1325, 28.

Antibiotics open a new field of research as food preservatives because they are a class that is relatively nontoxic. Experiments with canned pea puree showed that large amounts of subtilin reduced spoilage. Subtilin appears to kill spores only when they have begun to germinate. Addition of bicarbonate to otherwise complete media induces rapid germination of botulinum spores in anaerobic plates, but the addition of bicarbonate to subtilin-containing packs has not yet been found to enhance keeping quality. A big problem is a difference in resistance of strains to the antibiotic. With any one organism, the larger the amount of inoculum the greater the amount of subtilin needed to prevent spoilage. Whatever antibiotic is eventually considered for use, a decision will have to be made about the greatest number of spores that will have to be protected for.

Spilde, O., 1951, Antibiotics. Experiments with antibiotics in food preservation (in Norwegian). *Tidsskr. Hermetikind.* **37**, 297.

Subtilin, penicillin, streptomycin or chlortetracycline at 100 ppm did not successfully inhibit spoilage of fish dumplings in cans.

Williams, O. B. and Campbell, L. L., Jr., 1951, Effect of subtilin on thermophilic flat sour bacteria. *Food Research* **16**, 347; Abstr., 1951, Bacteriol. Proc. **51**, 16.

Thirty strains of obligate and facultative thermophilic flat sour bacteria were treated with subtilin and mild heat. Only one was affected by 5 ppm subtilin, 8 by 10 ppm, 19 by 15 ppm and 23 by 20 ppm; all were inhibited by 50 ppm.

Adams, A. T., Ayers, J. C., Tischer, R. G. and Ostle, B., 1952, Effect of subtilin on spoilage of thermal processed beef. *Food Technol.* **6**, 421.

High levels of subtilin with mild heat processing do not permanently inhibit germination of Putrefactive Anaerobe 3679 spores in comminuted beef. With drastic heat treatment and subtilin, extended inhibition is usually obtained.

Andersen, A. A., 1952, Effect of subtilin on spores of *Clostridium botulinum*. *J. Bacteriol.* **64**, 145.

Putrefactive Anaerobe 3679 and 6 cultures of *Cl. botulinum* were affected by subtilin. In broth containing subtilin which permitted rapid germination the number of viable spores of *Cl. botulinum* fell rapidly. If germination was repressed by deficiency of bicarbonate in the broth the number of viable spores fell slowly. Concentration of subtilin required to stop germination of spores went up as the number of spores was increased.

Burroughs, J. D. and Wheaton, E., 1952, Results of studies on the preservative action of antibiotics in processed food. *Austral. Food Mfr. & Distrib.* **7** (21), 16.

Evans, F. R. and Curran, H. R., 1952, Preserving action of subtilin and mild heat in normal and concentrated milk. *J. Dairy Sci.* **35**, 1101.

Tested normal and concentrated milks and inoculated some with resistant bacterial spores, processed with subtilin and heat at 100° for 10 minutes and observed over 5 to 8 months. Spoilage occurred consistently in milk inoculated with commercially important organisms, including *Cl. botulinum*, at relatively high subtilin levels.

Williams, O. B. and Fleming, T. C., 1952, Subtilin and the spores of *Clostridium botulinum*. *Antibiotics & Chemotherapy* **2**, 75.

The concentration of subtilin required for inhibition varied among strains and increased as the number of spores in the inoculum was raised. Spores incubated 3 days with 120 ppm subtilin, centrifuged and washed, promptly grew out on transfer; thus indicating that subtilin is sporostatic instead of sporicidal.

Andersen, A. A., Michener, H. D. and Oleott, H. S., 1953, Effect of some antibiotics on *Clostridium botulinum*. *Antibiotics & Chemotherapy* **3**, 52.

Thirty-two antibiotics were tested against *Clostridium botulinum* strain 62 A spores by Andersen's rapid plate technique.

Penicillin, oxytetracycline and chlortetracycline were effective at concentrations of 0.2 ppm; subtilin at 1 ppm; chloramphenicol, methylolgramicidin, and a crude preparation of cinnamycin at 5 ppm; and laterosporin, cinnamycin, actinomycin, thiolutin and nisin at 20 ppm. The following were ineffective at 20 ppm: bacitracin, circulin sulfate, comirin, endomycin, grifolin, grisein, hexahydrolupulon, lichenformin A5, lupulone, neomycin, netropsin, polymyxin D, polylysine, quercetin, streptomycin, streptothricin and subtenolin.

Denny, C. B., Bohrer, C. W. and Cameron, E. J., 1953, Effect of antibiotics on canned food spoilage organisms. A. C. S., Abstr. of Papers, 124th Meet., p. 31A.

The antibiotics tested were subtilin, tyrothricin, methylolgramicidin, rhatany root extract and avocado pit extract. Tyrothricin seemed to have greatest effect in accelerating rate of destruction of thermophilic flat sour spores. None significantly affected the rate of spore destruction of mesophilic putrefactive spoilage organisms.

Krasnow, I., Jann, G. J. and Salle, A. J., 1953, Effect of pH and heat on the activity of subtilin against spores of *Clostridium botulinum*. *Bacteriol. Proc.* **53**, 28.

Subtilin (1 ppm) inhibited 10,000 to 100,000 spores at either high (8.5 to 9.0) or low (5.5 to 6.5) pH, but only 1 to 10 spores at neutral. Heat had a detrimental effect at high or low pH but an additive effect at neutral pH.

LeBlanc, F. R., Devlin, K. A. and Stumbo, C. R., 1953, Antibiotics in food preservation. I. The influence of subtilin on the thermal resistance of spores of *Clostridium botulinum* and the Putrefactive Anaerobe 3679. *Food Technol.* **7**, 181.

The resistance to heat of Putrefactive Anaerobe 3679 spores suspended in pea puree containing 14.0 ppm of subtilin is about 47% of the resistance of these spores suspended in subtilin-free puree, and the resistance of *Cl. botulinum* spores is about 63%.

Lewis, J. C., Michener, H. D. and Stumbo, C. R., 1953, Antibiotics in the preservation of processed foods. A. C. S., Abstr. of Papers, 124th Meet., p. 30A.

The role of antibiotics in food preservation can be: prevention or retardation of the growth of viable spoilage organisms, killing of vegetable cells including germinating spores, and synergizing of the thermal death of spores of heat-resistant spoilage bacteria.

Michener, H. D., 1953, Bactericidal action of subtilin on *Bacillus stearothermophilus*. *Appl. Microbiol.* **1**, 215.

Both rough and smooth colonies are found in a culture of *B. stearothermophilus*. Subtilin does not affect the spores of the smooth type which become dormant quickly. The rough type spores germinate quickly and subtilin does affect the resulting vegetative cells. Subtilin shows no evidence of sporicidal action.

Michener, H. D., 1953, Effect of subtilin on ungerminated bacterial spores. *Bacteriol. Proc.* **53**, 29.

Subtilin, previously believed to have no effect on spores unless they have germinated or begun to germinate, seems to have a limited activity at low temperatures.

Varma, U. P., 1953, Antibiotics in food preservation. *Bihar Agr. Coll. Mag.* **4** (1), 29.

Stresses the need for other methods of canning and bottling foods, since the present high-heat treatment lowers the quality of the food. Discusses briefly the work of Drs. A. A. Andersen, H. D. Michener and A. Hirsch, using subtilin and mild heat in canned peas. Dr. Hirsch's experiments with nisin in cheese are also mentioned.

Kaufmann, O. W., Ordal, Z. J. and El-Bisi, H. M., 1954, The effect of several antibiotics on certain spore-forming organisms involved in food spoilage. *Food Research* **19**, 483.

Neomycin, celiomycin, streptin, circulin and five unidentified antibiotics were tested against five sporeforming organisms which cause spoilage of canned foods. In media, all except one of the unknown antibiotics were sporicidal for *Bacillus stearothermophilus*. Neomycin and celiomycin had a sporicidal effect on *B. thermoacidurans* and *Clostridium thermosaccharolyticum*. None was effective against *Cl. botulinum* or *Cl. sporogenes*.

Kaufman, O. W., Ordal, Z. J. and El-Bisi, H. M., 1954, The action of several antibiotics on the spores of *Bacillus thermoacidurans* in a tomato juice medium. *Food Research* **19**, 488.

Three antibiotics, celiomycin, M-2517-6 (911-A) and neomycin, were tested for activity against the spores of *B. thermoacidurans*, which causes flat sour spoilage in tomato juice. Both celiomycin and M-1517-6 (911-A) lost their

activity in the acid pH of tomato juice, and neomycin was inactivated by the mild-heat treatment used to destroy molds, yeast and vegetative bacteria in the canning industry.

Lewis, J. C., Michener, H. D., Stumbo, C. R. and Titus, D. C., 1954, Additives accelerating death of spores by moist heat. *J. Agr. Food Chem.* **2**, 298.

A discussion of the possible role of antibiotics in food preservation. Antibiotics may have real value for delaying the onset of spoilage in such foods as meat, fish and fresh vegetables. Retardation of food-poisoning bacteria and normal heat-resistant flora is another possible use. Low-acid or neutral foods such as vegetables and meats present a different problem because long-maintained preservation is desired rather than a delay in onset of spoilage.

Sixty-seven antibiotics were tested against Putrefactive Anaerobe 3679 at the level of 14 ppm and at 120.1°C; all were incubated for 90 days. Those giving positive results were: nigerium, oxytetracycline, erythromycin, penicillin G, chlortetracycline, subtilin, subtilin methyl ester, rhodomycin, chloramphenicol, tetracycline, thiolutin, cinnamycin, tyrothricin, laterosporin A, methylol gramicidin, polypeptin, nisin, catenulin, hexahydrolupulone, usnic acid.

Rittenberg, S. C., 1955, Can processing temperatures for non-acid canned foods be lowered? *Western Canner and Packer* **47**, 30.

This article discusses three new methods of food preservation: chemical additives (antibiotics), dielectric heating and sterilization by radiation. The section on antibiotics describes briefly the work of Andersen and Michener with subtilin and mild heat. Two questions have yet to be answered—

1. Exactly what is the action of antibiotics on the sporeforming organism?
2. Do sporeforming organisms develop resistance to antibiotics, as flies did to D.D.T.?

O'Brien, R. T., Titus, D. S., Devlin, K. A., Stumbo, C. R. and Lewis, J. C., 1956, Antibiotics in food preservation. II. Studies on the influence of subtilin and nisin on the thermal resistance of food spoilage bacteria. *Food Technol.* **10**, 352.

Spores of various food spoilage bacteria appear to be appreciably less heat resistant when heated in the presence of subtilin or nisin. Approximately 90 antibacterial substances were tested against P. A. 3679 but none was as effective as subtilin or nisin in reducing the thermal resistance of spores.

Rogacheva, A. I., 1956, Antibiotics and their utilization in the canning industry (in Russian). *Moskva, Pischepromizdat*, 88 pages.

Hawley, H. B., 1957, Nisin in food technology—1. *Food Manuf.* **32**, 370.

Bacteriological problems encountered in canned foods are discussed and the potential applications in canned goods—vegetables, tomato products, fruits, meat and meat products, fish and crustaceans, evaporated milk and milk products—are reviewed.

Hawley, H. B., 1957, Nisin in food technology—2. *Food Manuf.* **32**, 430.

The possible uses are described of nisin in the preservation of semipreserved meats, open-pack meats, sausages, pickles, beverages (sterilized and chocolate

milks), and cream and butter. Use of nisin also is discussed in combating the dangers of food poisoning.

Wheaton, E., Burroughs, J. D. and Hays, G. L., 1957, Flat sour spoilage of tomato juice and its control with subtilin. *Food Technol.* **11**, 286.

In a study to learn the effect of growth of flat sour spoilage organisms on the vacuum of canned tomato juice, it was concluded that the lower vacuum (av., 2.6 inches) of the inoculated lot after incubation appeared to be a result of bacterial activity rather than microleakage. Reports show the apparent effectiveness and stability of subtilin in acid media. This study also substantiated reports that subtilin appears to prevent off-flavor spoilage in tomato juice inoculated with spores of certain strains of *B. coagulans* (var. *thermoacidurans*). Subtilin at 5, 10 or 20 ppm was tested in conjunction with mild heat treatment.

D1. MEATS

Hounie, E., 1950, Meat preservation by antibiotics. *Food Manuf.* **25**, 508.

Tubes of inoculated meat treated with subtilin, tyrocidin, chloramphenicol, penicillin or streptomycin were left for 5 days at 5°C, giving the antibiotic a chance to penetrate into the muscular tissue. After the diffusion period the tubes were heated to 20°C and were kept under observation at this temperature for 10–15 days. By producing conditions that favored penetration of the antibiotic and at the same time avoiding the growth of bacteria by using low temperature, the meat was better preserved. The most satisfactory results were obtained with a combination of subtilin and streptomycin.

Goldberg, H. S., Lepovetsky, B. C., Weiser, H. H., Deatherage, F. E. and Kunkel, L. E., 1952, Studies on the microflora and deep tissues of beef, and their susceptibility to various antibiotics. *Bacteriol. Proc.* **52**, 17.

Ninety per cent of beef microflora studied showed marked susceptibility to chloramphenicol, chlortetracycline and oxytetracycline. The above antibiotics (0.5 to 2.0 ppm) doubled keeping time of ground beef. Penicillin, streptomycin and bacitracin were ineffective.

Hugli, E. and Prudent, I., 1952, Effect of subtilin and streptomycin on the formation of nitric oxide hemochromogen in cooked ground fresh beef. *Food Technol.* **6**, 129.

The red discoloration in cooked meats is attributed to nitric oxide hemochromogen which is formed from myoglobin and nitric oxide coming from the bacterial breakdown of nitrates. Subtilin at 400 ppm and streptomycin at 200 and 300 units per gram did not prevent formation of red discoloration in nitrate-treated meats, but streptomycin at 500 units per gram was effective.

Tarr, H. L. A., Southcott, B. A. and Bissett, H. M., 1952, Experimental preservation of flesh foods with antibiotics. *Food Technol.* **6**, 363.

Chlortetracycline, oxytetracycline and chloramphenicol in the order named proved the most effective inhibitors of growth of the natural mixed bacterial flora of fish and meat at temperatures between 0–21°C, while rimocidin inhibited yeast growth. Others used were streptomycin, penicillin, subtilin, polymyxin B, circulin, neomycin, bacitracin, gramicidin, methylol gramicidin, tyrothricin and an unnamed antibiotic.

Goldberg, H. S., Weiser, H. H. and Deatherage, F. E., 1953, Studies on meat: IV. Use of antibiotics in preservation of fresh beef. *Food Technol.* **7**, 165.

The storage life of ground beef was extended to 9 days at 10°C by the addition of 0.5 to 2.0 ppm of chloramphenicol, chlortetracycline and oxytetracycline. The controls and penicillin-, bacitracin- and streptomycin-treated samples spoiled in 5 days.

Gomutputra, C. and Fabian, F. W., 1953, Acids and chloramphenicol as sanitizing agents for meat contaminated with food-poisoning organisms. *J. Milk and Food Technol.* **16**, 220.

A 1% dipping solution of chloramphenicol was ineffective against food-poisoning staphylococci on the surface of pork and beef. Chloramphenicol was inferior to acetic, monochloroacetic, dehydroacetic (Na salt) and trichloroacetic acids against *Salmonellae*.

Tarr, H. L. A., Boyd, J. and Bissett, H. M., 1953, Experimental preservation of flesh foods with antibiotics. A. C. S., Abstr. of Papers, 124th Meet., p. 30A.

Chlortetracycline and oxytetracycline when incorporated into flesh foods caused a marked delay in growth of the indigenous microflora. Tests with tetracycline have indicated that it has no significant bacteriostatic activity when incorporated in flesh foods. Crushed ice containing chlortetracycline (1 ppm) was used in some of the experiments.

Weiser, H. H., Goldberg, H. S., Cahill, V. R., Kunkle, L. E. and Deatherage, F. E., 1953, Observations on fresh meat processed by the infusion of antibiotics. *Food Technol.* **7**, 495.

Internal spoilage (sours) in fresh meat must at present be prevented by chilling to an internal temperature below 60°F within 20 to 24 hours after slaughter. If chilling could be delayed, tenderness and perhaps juiciness and flavor might be improved.

Rounds and whole animals were infused with saline containing chlortetracycline so as to put approximately 2 ppm of the antibiotic into the meat. In the experiment with paired rounds, one round was infused while the other remained a control. Treated and control rounds were comparable in color, odor, taste and pH, although the infused round appeared a bit more moist. Seven out of 10 control rounds kept 48 hours at room temperature exhibited some off-color at some point; however, all of the infused rounds were sound.

Whole animals were infused, the carcasses split, and one side immediately chilled out whereas the other was allowed to stay at room temperature for 48 hours prior to chilling. Steaks from the sides kept at room temperature for 48 hours and then chilled out were as tender at 5 days postmortem as the others were at 2 weeks postmortem, and from the taster's point of view indistinguishable from normal meat. Chlortetracycline added to ground beef at 2 ppm showed no antibiotic left after 4 days at 10°C.

Weiser, H. H., Kunkle, L. E. and Deatherage, F. E., 1953, The use of antibiotics in meat processing. A. C. S., Abstr. of Papers, 124th Meet., p. 30A.

Broad-spectrum antibiotics (chlortetracycline, oxytetracycline and chloramphenicol) show promise in fresh meat processing. Antibiotics have no place in meat already populated with large numbers of bacteria and from present

information cannot be used to make old meat fresh. They have no place in overcoming improper and unsanitary meat processing practices. The purpose should be to enhance shelf-life and eliminate some distribution hazards.

Experiments on the use of antibiotics in fresh ground meat and infusion of carcasses prior to dressing out are reviewed. Public health aspects must be investigated especially concerning antibiotic residues in the meat.

Tarr, H. L. A., Boyd, J. W. and Bissett, H. M., 1954, Experimental preservation of fish and beef with antibiotics. *J. Agr. Food Chem.* 2, 372.

Spoilage of whole eviscerated fish was retarded markedly by ices containing 1 to 4 ppm of chlortetracycline, by holding 6 days at -1°C in sea water containing 2 ppm, or by one-minute immersion in solutions containing 50 or 100 ppm of the antibiotic prior to icing. In ground beef and fish chlortetracycline suppressed bacterial development but permitted yeast growth, and thiolutin (10 ppm) did not inhibit yeast development in the presence of chlortetracycline to any important extent. Puromycin was devoid of antibacterial activity.

Weiser, H. H., Kunkle, L. E. and Deatherage, F. E., 1954, The use of antibiotics in meat processing. *Appl. Microbiol.* 2, 88.

The work done in a previous paper is discussed and some new work is added on the effect of 2 ppm of penicillin, chloramphenicol and chlortetracycline on the microflora of ground beef. Gram-negative rods tend to overgrow all other bacteria in the meat; penicillin was ineffective against these but chloramphenicol and chlortetracycline retarded their development.

Downing, H. E., Hardie, W. B., McMahan, J. R. and Billman, D. C., 1955-56, Antibiotic preservation of meats. III. Intraperitoneal injection of oxytetracycline in sheep. *Antibiotics Ann.*, 734.

Intraperitoneal injections, 1 and 2 hours before slaughter, of oxytetracycline quarternary complex solubilized with tartaric acid and oxytetracycline hydrochloride produced comparable levels in blood and in leg, heart, liver, kidney and fat tissues in 16 sheep. Two control animals were compared with the 16 injected at levels of 1 and 3 milligrams of oxytetracycline activity per pound of body weight. One-half of each carcass was held for 7 days at room temperature (80° to 100°F). After 48 hours the control carcasses were spoiled, and after 5 days all treated carcasses gave off a moderately strong odor, but without a putrid smell and the flesh was not soft or gassy. Analysis of leg muscles of the treated carcasses showed an average of 68 per cent retention of antibiotic activity after 7 days at room temperature.

Downing, H. E., McMahan, J. R. and Baker, C., 1955-56, Antibiotic preservation of meats. IV. Intraperitoneal injection of oxytetracycline in hogs. *Antibiotics Ann.*, 737.

Oxytetracycline quarternary complex solubilized with tartaric acid and oxytetracycline hydrochloride (3 milligrams oxytetracycline activity per pound of body weight) were injected intraperitoneally into 4 hogs, 1 and 2 hours before slaughter. Satisfactory levels of antibiotic were produced in blood and tissues. One-half of each treated carcass and of 2 control animals were held at room temperature (80° to 100°F). Spoilage developed after 24 hours in the controls, and the treated carcasses remained in acceptable condition for 48 to 72

hours. Duplicate sides of one control carcass and of 2 treated animals were hung up and open surfaces were sprayed with a mixture containing 0.5% carboxymethyl cellulose, 10 ppm oxytetracycline and a wetting agent. The appearance of cut tissues of the sprayed animals was considerably better than the controls.

Ingram, M. and Barnes, E. M., 1955, Problems in the use of antibiotics for preserving meat. *J. Appl. Bacteriol.* **18**, 549.

Review of the use of antibiotics for preserving meat.

Kazakov, A. M. and Dyklop, V. K., 1955, Antibiotics for improving the stability of meat and meat products (in Russian). *Trudy, Vsesoyuz. Nauch.-Issledovatel. Inst. Myasnoi Prom.* **7**, 30.

The antibiotic effect of onion, garlic, gramicidin C, levomycetin, grizemin, syntomycin, ekmolin, chlortetracycline, eritron and antibiotic No. 551 on meat products was investigated. The onion, when freshly ground, had a significant antibiotic effect in ground meat. Pickled garlic was more effective than frozen garlic. Among the antibiotics, gramicidin C, grizemin and ekmolin were most effective in test cultures. In sausage levomycetin was best for holding down bacterial count but it was inactive for yeast and mold spores. The activity of levomycetin in sausage was significant at 100 mcg/gm, but at 200 mcg/gm the sausage had a bitter taste. Data are presented showing the efficiency of levomycetin in liver and meat sausages and in canned meats as affected by concentration, storage, temperature.

McMahan, J. R., Downing, H. E., Ottke, R. C., Luther, H. G. and Wrenshall, C. L., 1955-56, Antibiotic preservation of meats. I. Preliminary experiments with intraperitoneal injection of animals before slaughter. *Antibiotics Ann.*, 727.

Intraperitoneal injections of oxytetracycline quaternary complex solubilized with tartaric acid and oxytetracycline hydrochloride produced satisfactory blood and tissue levels in sheep. Eight lambs were injected with 6 mg oxytetracycline activity per pound of body weight 23, 11, 4 and 2 hours before slaughter and one lamb was used as a control. High antibiotic levels in the blood were maintained throughout the time period of 1 to 4 hours after injection. The highest tissue level was obtained 2 hours after injection.

Sacchi, E. M., McMahan, J. R., Ottke, R. C. and Kersey, R. C., 1955-56, Antibiotic preservation of meats. II. Intraperitoneal injection of oxytetracycline in beef cattle. *Antibiotics Ann.*, 731.

With one control animal, 4 bulls were injected intraperitoneally with oxytetracycline quaternary complex and 2 bulls with oxytetracycline hydrochloride, the former solubilized with tartaric acid. Using levels of 6, 3 and 1.5 grams oxytetracycline activity per 1000 pounds of body weight in the injection solutions one hour before slaughter, satisfactory levels of antibiotic were obtained in muscle tissue from each animal. Hind quarters of each animal were hung at room temperature (daytime temperature, 32° to 35°C) for 48 hours and the lowest tissue level of 0.5 ppm successfully inhibited putrefactive bacteria during the test period while the control spoiled. Tissue levels obtained with oxytetracycline hydrochloride were lower than those with the acidified

oxytetracycline quaternary complex, owing to the high alkalinity of the water used to make solutions.

Caporale, G., 1956, Antibiotics and ionizing radiation in the preservation of meat (in Italian). *Vet. ital.* **7**, 1042.

This is a short review article.

Dubrova, G. and Lazarev, E., 1956, Use of antibiotics in the meat industry (in Russian). *Miasnaia Indus. SSSR* **27** (2), 46.

Meat stored in solutions of Biomichina (soviet-produced chlortetracycline) of from 1 to 1000 mcg/ml were preserved for 2 to 3 days at 25° to 27°C, or 2 to 3 weeks at 2° to 3°C. Addition of allyl mustard oil improved the antibacterial effect and broadened the antibacterial spectrum. Small fish, such as anchovies and sardines, were also treated with good results. Additional satisfactory storage tests were run after dipping the meat or fish for one hour and then storing at 100% relative humidity at 5° and 25°C.

Jay, J. M., Weiser, H. H. and Deatherage, F. E., 1956-57, The effect of chlortetracycline on the microflora of beef, and studies on the mode of action of this antibiotic in meat preservation. *Antibiotics Ann.*, 954.

Fifteen long-cut beef rounds were hung at room temperature until some sign of deep spoilage occurred in a study to determine whether the infusion of beef carcasses with chlortetracycline brought significant alterations in the microbial flora, whether antibiotic-resistant strains emerged, and exactly how the antibiotic acts to retard spoilage of beef. One member of each of five pairs was infused postmortem with approximately 5 ppm of the antibiotic and the other five were from animals infused intraperitoneally at 7 ppm one or two hours before slaughter. From 14 of the rounds 358 isolates of bacteria were obtained and represented the following genera in the order of their frequency of isolation: *Proteus*, *Micrococcus*, *Streptococcus* (mostly enterococci), *Escherichia*, *Bacillus*, *Alcaligenes*, *Paracolobactrium*, *Corynebacterium*, *Streptomyces*, *Sarcina*, *Achromobacter*, *Flavobacterium*, *Pseudomonas* and *Clostridium*. Emergence of chlortetracycline-resistant mutants from previously sensitive strains of micrococci was not noted in the infused rounds. Some organisms with minimal inhibitory concentrations up to 100 mcg/ml chlortetracycline were inhibited in meat up to 48 hours by 5 ppm or less. The inhibition of *P. vulgaris* (MIC 50) in fresh beef by 5 ppm of the antibiotic was reversed by addition of Mn^{++} , and may be related to the multivalent cations in meat.

McMahan, J. R., 1956, Panel discussion. Food preservation, in Natl. Acad. Sci., Natl. Research Council. Proc. 1st Intern. Conf. on Use of Antibiotics in Agriculture, Washington, D. C., Publ. 397, 226.

Niven, C. F., Jr. and Chesbro, W. R., 1956-57, Complementary action of antibiotics and irradiation in the preservation of fresh meats. *Antibiotics Ann.*, 855.

Oxytetracycline, chlortetracycline and tetracycline were found to be equally effective in preventing spoilage of ground beef. A combination of oxytetracycline (10 ppm) and gamma radiation (10^5 rep.) delayed bacterial growth approximately twice as long as oxytetracycline treatment alone, and approximately three times as long as radiation treatment alone. Sorbic acid was

effective in retarding yeast and mold growth of treated meats. A combination of oxytetracycline, sorbic acid and gamma radiation was sufficiently effective so that microbial spoilage would no longer be the determining factor in the shelf-life of fresh meats.

Niven, C. F., Jr. and Chesbro, W. R., 1956, Antibiotics and irradiation in meat preservation. *Proc. Conf. on Research, Council on Research, Am. Meat Inst., Univ. Chicago, 8th Conf.*, 47.

The study concluded that, when good sanitation, low holding temperatures, radiation and antibiotics are combined, microbial spoilage will not be the determining factor in limiting the refrigerated life of fresh meat. Discoloration, caused by dehydration or other chemical causes, and "drip loss" will impair the desirable appearance of the displayed meat. Experiments continue with meat cuts of near retail size to evaluate these nonbacterial changes.

Ordal, Z. J. and Brown, W. L., 1956-57, The effect of oxytetracycline on the keeping quality of cured hams. *Antibiotics Ann.*, 860.

The combination of oxytetracycline (5 mcg/gm) and salt was more effective in preventing spoilage of ground fresh lean pork than was either agent alone. The effect of oxytetracycline was most pronounced when salt concentrations were low. The addition of the antibiotics to curing solutions (4% salt) markedly extended the storage life of hams.

Cahill, V. R., 1957, Antibiotics in the preservation of fresh meat. *J. Am. Dietet. Assoc.* **33**, 30.

Surface spoilage of meat is a problem and frequently a deterrent to efficient merchandising. Although the antibiotics discussed are not bactericidal, they are effective in inhibiting the growth of many organisms found in and on fresh meat. Color and flavor of meat are not changed by the use of appropriate antibiotics. These antibiotics disappear from meat during a four- or five-day storage period, and they are largely destroyed by cooking temperatures. Evidence is not yet available to indicate that resistant strains of bacteria will develop or that sensitive organisms become less sensitive when these antibiotics are used. Antibiotics complement and are not a substitute for strict sanitation and adequate refrigeration in the processing and distribution of meat.

Ginsberg, A., Hill, E. C. and Grieve, J. M., 1957, Oxytetracycline and its use as a meat preservative in underdeveloped countries. *Vet. Record* **69**, 983.

Fifteen cattle and 13 sheep were treated with oxytetracycline using intraperitoneal injections, and spray and dipping methods. The carcasses were tested after 24, 48 and 72 hours under varying and severe tropical or semitropical conditions. Use of the antibiotic improved the keeping quality of fresh meat and, particularly, beef by extending its fitness for human consumption 48 to 72 hours when the initial handling of slaughter animals conformed with the demands of meat hygiene. The intraperitoneal method, although most effective in controlling deep spoilage, requires too much time under commercial conditions. Dipping of carcasses is suitable for small stock only and is unsuitable for commercial slaughter. The spray method, in its over-all effect equal to the intraperitoneal and more efficient in controlling surface spoilage, is suggested as a routine measure for the preservation of fresh meat in tropical and semitropical countries.

Jay, J. M., Weiser, H. H. and Deatherage, F. E., 1957, Further studies on the preservation of beef with chlortetracycline. *Food Technol.* **11**, 563; Abstr., 1957, *Food Technol.* **11**, 26.

Low concentrations of chlortetracycline inhibited resistant strains of *Proteus*, *Pseudomonas* and strains of certain other genera in beef but not in complete nutrient media. Oxytetracycline and tetracycline were similarly effective, but streptomycin, chloramphenicol and penicillin did not act similarly against the same organisms. Significant inhibition of strains of *Proteus vulgaris* by 5 ppm chlortetracycline in beef were observed with inocula as high as 65,000 per gram. Of a group of resistant bacteria commonly associated with beef 70% were inhibited by 5 ppm chlortetracycline. Effectiveness of the tetracycline antibiotics in retarding spoilage in meats may be related to their ability to disturb the ionic balance in the product.

Jay, J. M., Weiser, H. H. and Deatherage, F. E., 1957, Studies on the mode of action of chlortetracycline in the preservation of beef. *Appl. Microbiol.* **5**, 400.

Previous study showed that certain chlortetracycline-resistant strains of *Proteus vulgaris* which required chlortetracycline at concentrations as high as 100 ppm for inhibition in broth, were actually inhibited in beef for 24 to 48 hours by as little as 3 ppm. It was found that the inhibition in beef by the sub-bacteriostatic concentration of chlortetracycline was reversed by adding Mn^{++} ions to the meat. The hypothesis was advanced that chlortetracycline competed with the organisms for essential ions. In this study none of the organic compounds (B vitamins, amino acids, purine and pyrimidine bases, and Krebs' cycle intermediates) was effective in reversing the chlortetracycline inhibition of chlortetracycline-resistant *Proteus* in beef. The following inorganic compounds were effective and are listed in the order of their effectiveness: tungstate, molybdate, magnesium, calcium, borate, silicate, strontium and barium.

Krücken, J., 1957, Use of antibiotics in the preservation of foods, especially meat (in German). - *Arch. Lebensmittelhyg.* **8**, 16.

General article which gives methods of using antibiotics in preserving meat. Effects of long-term ingestion by humans of these compounds are reviewed.

Moreno Calvo, J. and Pozo Fernandez, R., 1957, Antibiotics and refrigeration in meats (in Spanish). *Rev. frio* **2**, 223.

A short review is given of the various techniques in combination with refrigeration which have been used in meat preservation, including poultry.

Sacchi, E. M., McMahan, J. R., Ottke, R. C. and Wrenshall, C. L., 1957, New methods of preslaughter administration of antibiotics. Presented 17th Ann. Meet., Inst. Food Technologists; Abstr., 1957, *Food Technol.* **11**, 26.

New antemortem injection techniques including tail injection and intrathoracic injection, and refinements of the intraperitoneal injection are described. Influence of other agents used in antibiotic formulations on blood and tissue concentrations is reported.

Tellez Roldan, R., 1957, Preservation of meat with antibiotics (in Spanish). *Rev. nacl. agr.* **51**, 40.

The author emphasizes that commercial use of chlortetracycline in beef preservation is not authorized in Colombia. The infusion method cannot be carried out by most slaughterhouse employees in that country, and serious consideration of authorization should await the approval of the U. S. government.

Brown, P. D., Ginger, B., Chesbro, W. R., Weir, C. E. and Wilson, G. D., 1958, Use of antibiotics and gamma irradiation in the aging of steaks at 110°F. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 46.

In studies of the high-temperature aging of beef round steaks, 20 to 30 ppm oxytetracycline and 40 to 45 thousand rad of irradiation were used as a preservative. Results from round steaks of eight animals showed that 40 to 45 thousand rad gamma irradiation did not affect the tenderizing process when steaks were aged at 110°F. In another experiment eight pairs of inside U. S. Utility rounds and eight pairs of U. S. Good rounds were infused with 20 to 30 ppm oxytetracycline, respectively, and cut into steaks. Control steaks were frozen for comparison with adjacent steaks aged 16, 24 and 40 hours at 100°F, which were frozen prior to serving to the taste panel. A significant increase in tenderness occurred after aging for 24 hours, with no further increase after 40 hours. The effects of short time-high temperature aging of beef also were studied.

Cain, R. F., Anderson, A. W. and Malaspina, A. S., 1958, Effect of irradiation on antibiotic-treated meats. *Food Technol.* **12**, 582.

Chlortetracycline, oxytetracycline and tetracycline at levels of 5 and 50 ppm were added to ground beef and distilled water and irradiated. At radio-pasteurization dosages (0.18 to 0.72 megarad) the antibiotics retained sufficient activity to offer protection to meats during storage. Intrathoracic injection of hogs with oxytetracycline before slaughter produced a meat with superior keeping quality when compared with controls. Meat cuts from oxytetracycline-injected animals when exposed to radio-pasteurization dosages had more than a threefold extension in storage life at 50°F. Beef slices dipped in a 100-ppm oxytetracycline solution and irradiated at 0.1 megarad showed a more pronounced effect of the complementary action of the two methods.

Matsui, T., Tokutomi, G., Takase, A., Akao, Y., Sano, R. and Bito, B., 1958, Prevention of meat spoilage by use of oxytetracycline (in Japanese). *Bull. Inst. Public Health, Tokyo*, in press.

With comparable controls, one castrated bull was injected by the intraperitoneal route and a tail injection was made in one beef cow, using a level of 3 grams oxytetracycline activity per 1000 pounds of body weight in the injection solutions. Two hours after injection the animals were slaughtered and samples of blood, chuck, rib, round, heart and liver were selected for storage with controls at 30°C, at room temperature and under refrigeration. Samples of chuck, round, heart and liver from a milk cow were dipped in an oxytetracycline solution (100 ppm), and also were stored with samples from a control animal under the same conditions. Organoleptic tests, antibiotic tissue levels, deep tissue counts of aerobic bacteria and Clostridia, and measurements of pH, volatile basic nitrogen and amino nitrogen were made at intervals during storage. Increases in aerobic bacteria were greater in control samples, and

differences were particularly significant in samples stored at high temperatures. Numbers of Clostridia in antibiotic-treated samples did not show a significant increase throughout the experiment, regardless of storage conditions in contrast to controls. Differences in pH value, volatile basic nitrogen and amino nitrogen were prominent between antibiotic-treated samples and controls. Except for high levels in liver samples from injected animals, the tissue level of antibiotic throughout the study was 1 to 2 mcg/gm.

D2. POULTRY

Kohler, A. R., Miller, W. H. and Broquist, H. P., 1955, Aureomycin, chlortetracycline and the control of poultry spoilage. *Food Technol.* 9, 151.

Of ten antibiotics tested—chloramphenicol (10 ppm), erythromycin (10 ppm), procaine penicillin (10 ppm), neomycin (10 ppm), chlortetracycline (10 ppm), streptomycin (10 ppm), carbomycin (10 ppm), polymyxin B (100 units per ml), bacitracin (11 units per ml), actidione (10 ppm)—chlortetracycline proved best in controlling growth of microflora of spoiling chicken.

Ziegler, F. and Stadelman, W. J., 1955, The effect of Aureomycin treatment on the shelf life of fresh poultry meat. *Food Technol.* 9, 107.

An experiment was run dipping chicken halves into 10, 20 and 40 ppm chlortetracycline for 10 minutes, draining, packaging in polyethylene bags and refrigerating at 2.2°C. Spoilage was determined by slime and odor. Off-odor of control was noticed at 11.6 days, of 10 ppm at 17.6 days, of 20 ppm at 18.4 days and of 40 ppm at 18.9 days.

Abbey, A., Darken, M. A., Kline, E. F., Kohler, A. R., Maturi, V. F., Miller, W. H. and Upham, S. D., 1956-57, Comprehensive studies of the use of a food grade of chlortetracycline in poultry processing. III. Evaluation of raw and cooked poultry by microbiological chlortetracycline assay. *Antibiotics Ann.*, 831.

Whole chickens were held in chlortetracycline solutions (10, 30 and 300 ppm) 2 and 24 hours. Microbiological assay of various muscles of raw and cooked poultry showed that raw tissue concentrations of chlortetracycline were increased with solutions of higher concentrations. Chickens, when boiled, roasted, broiled and fried, had no chlortetracycline residues except those held for 24 hours in a 300-ppm solution. Chickens dipped in the 300-ppm solution lost more than 98 per cent of chlortetracycline content during cooking.

Ayres, J. C., Walker, H. W., Fanelli, M. J., King, A. W. and Thomas, F., 1956, Use of antibiotics in prolonging storage life of dressed chicken. *Food Technol.* 10, 563.

Chlortetracycline, oxytetracycline, tetracycline, streptomycin, neomycin, aerosporin, rimocidin, A-5288 (Lederle), mycostatin and ascosin were tested for antimicrobial effects in dipping solutions for poultry. Chlortetracycline and oxytetracycline were the most effective. Graphs of bacterial count vs. days of storage at 4.4°C indicate that chlortetracycline is more effective than oxytetracycline at 3 ppm but that the two antibiotics are comparable at 30 ppm.

With concentrations of chlortetracycline above 3 ppm, no direct relationship was shown between the amount of antibiotic used and the number of organisms

found on the surface of the birds. For example, relative bacteriostatic action of the various levels of chlortetracycline were $30 > 90 > 270 > 3 > 10 > 1 > 0$ at 7 days and $30 > 10 > 90 > 270 > 3 > 1 > 0$ at 18 days.

After two days' storage, 65% of the flora on the controls and 95% on the chlortetracycline-treated birds were of the *Pseudomonas-achromobacter* type. On continued storage the percentage of these organisms on the control and treated birds approached the same figure but the total population of the controls was more than ten times that of the treated birds.

Broquist, H. P., Kohler, A. R. and Miller, W. H., 1956, Retardation of poultry spoilage by processing with chlortetracycline. *J. Agr. Food Chem.* **4**, 1030.

When eviscerated chickens were dipped for 2 hours in chlortetracycline solutions (1 to 20 ppm), bacteriostatic amounts of the antibiotic were found in breast muscles; however, such amounts were not obtained by feeding chlortetracycline in concentrations as high as 1000 ppm to chickens before killing. Chickens processed with the antibiotic (10 to 15 ppm) in the chill tanks under commercial conditions remained fresh and edible significantly longer than control birds. Shelf-life of poultry about to spoil was not extended by dipping in chlortetracycline solutions.

Eklund, M. W., Spencer, J. V., Sauter, E. A. and George, M. H., 1956, The effect of different methods of chlortetracycline application on the shelf-life of chicken fryers. Presented 45th Ann. Meet., Poultry Sci. Assoc.; Abstr., 1956, *Poultry Sci.* **35**, 1141.

Effects of chlortetracycline in ice, as a spray, and as a dip were studied in fresh chicken fryers held at 32° to 34°F. All concentrations in ice (1, 5, 10 and 15 ppm) increased shelf-life approximately four days. Birds treated at 1 and 5 ppm showed a predominant flora of gram-negative rods, and yeasts and molds predominated in 10- and 15-ppm birds. Shelf-life was increased approximately five days in both cut-up and whole eviscerated fryers sprayed for 10 seconds (100 ppm). All birds dipped in a 100-ppm solution (7, 10, 15 and 30 seconds) showed an increase in shelf-life of approximately six days. In sprayed and dipped birds the predominant flora was yeasts and molds.

Harms, J., 1956, The antibiotic revolution. *Poultry Process. Market.* **62**, 20.

Use of antibiotics to prolong freshness of poultry is expected to start a big trend toward tray-packaging of cut-up poultry at the plant. The giblet problem also may be solved.

Hines, L. R., 1956, Panel discussion. Food preservation, in Natl. Acad. Sci., Natl. Research Council. Proc. 1st Intern. Conf. on Use of Antibiotics in Agriculture, Washington, D. C., Publ. 397, 227.

Brief data on pick-up of chlortetracycline by chicken tissues dipped in chlortetracycline solutions and the effect of boiling on residual levels of the antibiotic.

Kohler, A. R., Abbey, A., Darken, M. A., Firman, M. C., Kline, E. F., Maturi, V. F., Miller, W. H. and Upham, S. D., 1956-57, Comprehensive studies of the use of a food grade of chlortetracycline in poultry processing. II. Relation of microbial counts of freshness. *Antibiotic Ann.*, 822.

Poultry processed in chill tanks employing in-plant chlorinated water had better shelf-life than control birds but not as good as birds treated with chlortetracycline.

Freshly killed poultry were chilled in tanks with and without added antibiotic for periods ranging from 2 to 24 hours. The bacterial load of stored poultry from the chlortetracycline-treated birds was almost independent of chill time. In contrast the control birds had bacterial loads that increased sharply as immersion time was lengthened.

The application of chlortetracycline to the cut-up parts of chickens gave slightly better results than antibiotic treatment of the whole chicken followed by cutting-up; either method was much superior to the untreated cut-up controls. Treatment methods for giblets and the effect of storage temperatures are also discussed.

Miller, W. H., 1956, Antibiotic introduced as spoilage inhibitor for fresh poultry. *Food Eng.* **28**, 43.

Tolerance level allowed in the United States for chlortetracycline in uncooked poultry is discussed with a presentation of experimental work in other foods.

Shannon, W. G. and Stadelman, W. J., 1956, Aureomycin in the control of bacterial spoilage of eviscerated poultry at different temperatures. Presented 45th Ann. Meet., Poultry Sci. Assoc.; Abstr., 1956, *Poultry Sci.* **35**, 1170.

Control half-birds were dipped for 15 minutes in water and the other half was dipped for 15 minutes in chlortetracycline solution (10 ppm). Each half was enclosed in a gas-proof plastic bag and stored at 32°, 37°, 42°, 47° or 68°F. Spoilage was measured by taking a daily smear from each half-bird and by noting the presence of odor and slime. Positive slides appeared sooner on the controls and at the higher temperatures. Evaluation by slime and odor showed a similar relationship. However, at high temperatures they appeared almost simultaneously with positive slide, while at 37° and 32° they appeared one to five days after positive slide.

Spencer, J. V., Eklund, M. W., Sauter, E. A. and Hard, M. M., 1956, The effect of different packaging materials on the shelf-life of antibiotic treated chicken fryers. Presented 45th Ann. Meet., Poultry Sci. Assoc.; Abstr., 1956, *Poultry Sci.* **35**, 1173.

Chicken fryer halves were dipped in solutions of 5 and 10 ppm chlortetracycline for 10 and 20 minutes. The half-birds and controls were packaged in cellophane, polyethylene, evacuated heat shrinking polyethylene, and evacuated heat shrinking polyvinylidene, and stored at 32°F until spoilage. The shelf-life of untreated control birds packaged in both evacuated heat shrinking films showed an increase of approximately two days over those packaged in cellophane or polyethylene. Shelf-life increased as antibiotic concentration or dipping time was increased, and it appears that the less permeable polyvinylidene film offers an unfavorable atmosphere for the growth of yeasts and molds. There was little difference in shelf-life of birds packaged in cellophane or nonevacuated polyethylene.

Stadelman, W. J., 1956, Several factors are responsible for increasing shelf life of unfrozen poultry. *Am. Poultry J.* **87**, 28.

High sanitary standards and low storage temperatures must be maintained to get maximum benefit from the application of antibiotics to poultry. At storage temperatures between 30° and 40°F, a day is added to the shelf-life for each degree that the temperature is lowered. Other important factors for increasing shelf-life are discussed.

Stadelman, W. J., Marion, W. W. and Eller, M. L., 1956-57, Antibiotic preservation of fresh poultry meat. *Antibiotics Ann.*, 839.

The effectiveness of chlortetracycline and oxytetracycline in inhibiting spoilage of fresh chicken meat was tested with 112 chickens. Use of either antibiotic significantly increased the shelf-life. Dipping of parts of chicken in antibiotic solutions did not increase the shelf-life beyond that of birds dipped prior to cutting up. There were no statistically significant differences in shelf-life of birds treated with oxytetracycline or chlortetracycline when held at 41°F.

Windlan, H., Abbey, A., Darken, M. A., Firman, M. C., Kline, E. F., Miller, W. H. and Upham, S. D., 1956-57, An investigation of the process using food grade chlortetracycline as applied to turkeys. *Antibiotics Ann.*, 849.

Turkeys were commercially treated in slush-ice tanks with solutions containing 10 and 30 ppm chlortetracycline for 2 and 22 hours. One group of birds were refrigerated at 35°F and the second group was frozen for 30 days. Each group was divided in two lots, one for microbial count and one for chlortetracycline assay. Birds used for chlortetracycline assay were sampled raw on one side, then cooked and sampled on the opposite for residues after cooking.

Shelf-life of turkeys iced with 10 ppm chlortetracycline for 2 hours and refrigerated was greatly extended. After 10 days control birds in this group were spoiled, while in untreated birds shelf-life was extended 7 to 10 days. Turkeys frozen for 30 days were thawed and treated birds (at 10 ppm) were acceptable 22 days after thawing; control birds were spoiled.

Wrenshall, C. L. and McMahan, J. R., 1956, How newly ok'd antibiotic boosts poultry shelf-life. *Food Eng.* 28, 53.

The U. S. Food and Drug Administration has approved the use of oxytetracycline, a broad-spectrum antibiotic, for poultry processing. A tolerance of 7 ppm for residues of this antibiotic in or on uncooked poultry has been established. Oxytetracycline is unstable at cooking temperatures and residues in poultry, as eaten, are undetectable. Principal use of oxytetracycline is in retarding spoilage of whole birds. These birds are chilled in an ice bath containing a special oxytetracycline product which will extend the freshness time 50 to 100%. However, it is noted that the use of antibiotics will not in any way lessen the need for proper plant sanitation or good processing and distribution methods. Furthermore, they are of no use in concealing spoilage that has already occurred or in reclaiming foods that are heavily contaminated. Also refrigeration is still essential for maximum success in the performance of this agent.

Carlin, A. F., Holl, B. E. and Walker, H. W., 1957, Correlation between flavor and number of microorganisms associated with eviscerated chicken treated with chlortetracycline. *Food Technol.* 11, 573; Abstr., 1957, *Food Technol.* 11, 19.

One day after slaughter whole birds were immersed in a 10-ppm solution of antibiotic at 50°F for 30 minutes. After treatment the birds were cut up and packaged in Cryovac and LSAD-300 cellophane, and bacterial and organoleptic evaluations were made at regular intervals up to 11 days of storage. Flavor tests were made by a taste panel on deep-fat fried samples. Meat flavor scores showed that as storage time increased a gradual deterioration in chicken quality occurred regardless of packaging or antibiotic treatment. The preservative effect of the antibiotic treatment was limited to inhibiting the growth of the bacteria since the flavor of the treated and untreated samples did not differ significantly. Other undetermined factors may be equally or more important than bacterial counts in determining the flavor of stored poultry.

Darcel, A. P., 1957, Approved antibiotics will mitigate poultry perishability problem. *Can. Food Inds.* **28**, 19.

Government approval has been made for use of chlortetracycline and oxytetracycline in fish and poultry in Canada and also for use of both antibiotics in fresh poultry in the United States. Their use does not in any way substitute for proper food plant sanitation nor can it reclaim already-spoiled foods. Extension of shelf-life of poultry is welcomed by Canadian marketers because it will mean greater volume of sales and taking full advantage of market conditions anywhere in Canada. Consumption of poultry in Canada is up, and the number of broilers raised in Ontario tripled in the past four years.

Ng, H., Vaughn, R. H., Stewart, G. F., Nagel, C. W. and Simpson, K., 1957, Antibiotics in poultry meat preservation: Development of resistance among spoilage organisms. *Appl. Microbiol.* **5**, 331.

During several months in 1956 and 1957 samples of birds from a processing plant and retail stores were stored at 45°F until spoiled. Organisms isolated were studied and data showed that resistant forms prevailed in chickens treated with the antibiotic and in chickens not treated but from a plant in which a regular portion of the poultry was treated with chlortetracycline. Resistant forms were not found on spoiling poultry which had been processed in plants not using the antibiotic.

Njoku-Obi, A. N., Spencer, J. V., Sauter, E. A. and Eklund, M. W., 1957, A study of the fungal flora of spoiled chlortetracycline treated chicken meat. *Appl. Microbiol.* **5**, 319.

Half-birds were immersed in a 20-ppm chlortetracycline solution for 10 minutes, packed in polyethylene bags and stored at 34° to 36°F. The presence of slime and spoilage odor was used as indications of spoilage. Serial dilutions of 1/10, 1/100 and 1/1000 of rinse water from spoiled halves were plated to study flora. Yeasts isolated from treated meat by quantitative methods (viable cells per gram of carcass) were *Saccharomyces cerevisiae* (800), *Saccharomyces dairensis* (560), *Rhodotorula minuta* (375), *Geotrichum candidum* (340), *Torulopsis holmii* (300), *Candida parapsilosis* (240), *Torulopsis globosa* (180) and *Candida guilliermondii* (175). Those from untreated controls were *Rhodotorula minuta* (600), *Geotrichum candidum* (410) and *Torulopsis albida* (165). The molds isolated from treated meat were *Cladosporium*, *Mucor*, *Penicillium*, *Rhizopus nigricans*, *Torula nigra* and *Trichoderma*. Those isolated from controls were *Alternaria*, *Aspergillus*, *Mucor* and *Rhizopus*. The potential

pathogenicity of *Candida parapsilosis* and the advisability of precautionary measures were discussed.

Shannon, W. G. and Stadelman, W. J., 1957, The efficacy of chlortetracycline at several temperatures in controlling spoilage of poultry meat. *Poultry Sci.* **36**, 121.

With controls, half-birds were dipped 15 minutes in chlortetracycline solutions (10 ppm) and stored at 32°, 37°, 42°, 47° and 68°F. Observations for spoilage were made daily using a rapid qualitative microscopic method and appearance of slime and off-odor. Improvement of storage life in controls and treated birds was progressively greater as the storage temperature was reduced from 68° to 32°F, the differences in storage times being significant at all temperatures.

Shrimpton, D. H., 1957, The use of chlortetracycline (Aureomycin) to retard spoilage of poultry carcasses. *J. Sci. Food Agr.* **8**, 486.

Under conditions approaching those used in the United States use of chlortetracycline in poultry processing was compared with results obtained with some of the features of present British practice. American results were confirmed but chlortetracycline failed to extend the storage life of eviscerated poultry carcasses in the absence of refrigeration. When stored at 15°C, chlortetracycline carcasses showed an unusual type of spoilage. Extensive growth of yeasts and molds was a striking demonstration of the rapid growth of a microflora resistant to chlortetracycline and, under traditional methods, suppressed by growth of the typical bacterial spoilage flora. Presence of this growth in the later stages of storage raised doubts as to whether there had also been a growth of some strain of bacteria which are known to be resistant to chlortetracycline, such as the *Salmonellas*, and which could multiply at 15°C.

Vaughn, R. H., Nagel, C. W., Sawyer, F. M. and Stewart, G. F., 1957, Antibiotics in poultry meat preservation: Comparison of the tetracyclines. Presented 17th Ann. Meet., Inst. Food Technologists; Abstr., 1957, *Food Technol.* **11**, 26.

Effectiveness of chlortetracycline, oxytetracycline and tetracycline in the preservation of chilled poultry was studied under commercial conditions. Eviscerated fryers were chilled for two hours in ice slush containing 20 ppm of the antibiotic. Some fryers were cut up, tray-packed and wrapped in plastic and whole birds were packaged in polyethylene bags. Antibiotic-treated and control birds were stored at 45°F; additional control birds were held on ice (32°F). Tray-packed fryers treated with chlortetracycline, oxytetracycline and tetracycline and controls stored at 45°F showed definite off-odor at an average of 7.25, 6.5, 7.5 and 6.25 days, respectively. Whole birds were judged spoiled at 11.5, 9.0, 8.5 and 8.0 days, respectively. In general, total psychrophilic bacterial counts were an unreliable index of organoleptic quality.

Vaughn, R. H., Nagel, C. W., Sawyer, F. M. and Stewart, G. F., 1957, Antibiotics in poultry meat preservation: A comparison of the tetracyclines. *Food Technol.* **11**, 426.

Oxytetracycline, chlortetracycline and tetracycline were compared for effectiveness in improving the storage life of chilled poultry. Under com-

mercial conditions, the birds were chilled for two hours in ice slush containing 20 ppm of antibiotic. In four trials the birds were cut up and tray packed and in two trials the birds were packaged whole in polyethylene. All birds were stored at 7.2°C (45°F). Oxytetracycline treatment increased storage life in three of the six trials, chlortetracycline in five trials, and tetracycline in all six trials. Increases in storage life ranged from 0 to 3 days. Whole birds kept much better than those which had been cut up.

Wells, F. E., Fry, J. L., Marion, W. W. and Stadelman, W. J., 1957, Relative efficacy of three tetracyclines with poultry meat. *Food Technol.* **11**, 656; Abstr., 1957, *Food Technol.* **11**, 26.

Tetracycline, oxytetracycline and chlortetracycline were compared at four storage temperatures (0°, 3°, 9° and 12°C) for extension of shelf-life of poultry meat. After chilling and prior to packaging, half-birds were immersed in a solution of antibiotic (10 ppm) for 15 minutes. Effectiveness of all antibiotics was greatest at the lower temperatures. Under all conditions chlortetracycline was more effective in prolonging storage time than oxytetracycline or tetracycline. In poultry processed at a commercial plant there was an increase at 9° and 12°C in organisms belonging to the *Flavobacterium* group. Increases in yeasts were observed in the antibiotic-treated birds.

Yacowitz, H., Pansy, F., Wind, S., Stander, H., Sassaman, H. L., Pagano, J. F. and Trejo, W. H., 1957, Use of nystatin (Mycostatin) to retard yeast growth on chlortetracycline-treated chicken meat. *Poultry Sci.* **36**, 843; Abstr., 1956, *Poultry Sci.* **35**, 1181.

Increased yeast growth and yeasty odor were found in chlortetracycline-treated chicken parts. The suppression of bacterial growth by use of the broad-spectrum antibiotic results in increased growth of yeasts and molds. Nystatin at levels of 5 and 10 ppm, when added to dip solutions containing 10 ppm chlortetracycline, effectively prevented the growth of yeasts and molds and the development of fungal odors on stored chicken wings and drumsticks. Assays showed that nystatin did not penetrate chicken skin or muscle and was rapidly inactivated by boiling.

Anderson, G. W., Epps, N. A., Snyder, E. S. and Slinger, S. J., 1958, Comparative effectiveness of feeding Aureomycin and dipping in an Aureomycin solution as a means of preserving poultry meat. *Poultry Sci.* **37**, 174; Abstr., 1956, *Poultry Sci.* **35**, 1130.

In general, organoleptic examination indicated that both the feeding of chlortetracycline (1000 or 2000 grams per ton of diet) and dipping in a chlortetracycline solution (10 ppm) delayed the production of off-odors and slime. Birds in both groups were stored at 3°C and retained their keeping quality about 28 days. Only minor improvement in keeping quality resulted with dipping birds when they had been fed the antibiotic.

Essary, E. O. and Moore, W. E. C., 1958, Influence of scald temperatures, chill times, and holding temperatures on the bacterial flora and shelf-life of freshly chilled, tray-pack poultry. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 52.

Fryers were processed in the laboratory and in two commercial plants to determine the influence of scald temperatures, length of chill time, and holding

temperatures on the shelf-life of tray-pack birds. Comparisons were also made on the shelf-life of untreated fryers with that of birds treated with chlortetracycline using different chill times and holding temperatures. Bacterial counts were made from skin samples of two birds, each scalded at 128°F for 50 seconds and at 138°F for 30 seconds and held at 36°F. There was little, if any, difference in the types of bacteria found on these fryers. There was significant difference in the shelf-life of fryers, treated and untreated, when chilled for different lengths of time and held at different temperatures.

Frye, G. R., Weiser, H. H. and Winter, A. R., 1958, Relative effectiveness of increasing shelf life of poultry meat by long and short periods of antibiotic feeding. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 52.

One, three or five days before slaughter birds were maintained on a basal diet supplemented with 1000 ppm chlortetracycline. An additional group of birds received the supplemented diet for several months. Birds were processed and stored at 35°F with control samples and samples dipped in 10 ppm chlortetracycline. There was a parallel relationship in the number of bacteria present between the five-day feeding and the antibiotic dip samples. Shelf-life of the birds maintained on prolonged antibiotic feeding did not vary significantly from that of the controls.

Kraft, A. A., Elliott, L. E. and Brant, A. W., 1958, Effect of antibiotic treatment on storage life of turkeys. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 52.

Action of chlortetracycline and chlorine on turkey meat was studied. Birds treated with chlortetracycline were held overnight in the antibiotic solution in chill tanks; some were dipped again in the antibiotic after evisceration and before packaging. Chlorine treatment was made on other birds in separate tanks. Chlortetracycline gave an increased storage life of 7 to 14 days when used as a single dip. With a second dip in antibiotic solution and ice after evisceration, keeping time was further increased. Chlorine treatment produced a decrease in bacterial numbers on the skin surface during the first week of storage, but was not as effective as the antibiotic in retarding storage.

McVicker, R. J., Dawson, L. E., Mallmann, W. L., Walters, S. and Jones, E., 1958, Effect of certain bacterial inhibitors on shelf-life of fresh fryers. *Food Technol.* **12**, 147; Abstr., 1957, *Food Technol.* **11**, 25.

Effects of chlorine, chlortetracycline and oxytetracycline on the shelf-life of fresh fryers were studied. Commercially produced half-birds were immersed for four hours in ice water, in ice water plus 10 ppm chlortetracycline, in ice water plus 10 ppm oxytetracycline and in ice water plus 20 ppm chlorine. Fryers treated with chlortetracycline had lower bacterial counts and a longer shelf-life, as evaluated by raw odors and cooked flavor. Cooked flavor differences between treatments were less noticeable. Odor and flavor scores of the chlortetracycline-treated birds were lower than controls the first four days after processing, but were higher the final week. Oxytetracycline and chlorine were less effective than chlortetracycline in extending shelf-life of fryers.

Meyer, R. C., Winter, A. R. and Weiser, H. H., 1958, Edible protective coatings for extending the shelf-life of poultry. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 50.

To determine the value of edible nontoxic coatings in retarding microbial spoilage of cut-up and eviscerated half-birds, the coating materials were used with and without a conventional wrapper, and in conjunction with various antibiotics at storage temperatures of 75°, 55° and 35°F. The coating materials did not alter the appearance of the samples and were most effective in combination with one or more of the antibiotics. A comparison of the growth rates of the microflora showed that these organisms multiply faster on the skin than on the visceral surface. Samples coated with a material containing an antibiotic had a longer shelf-life than those processed with an antibiotic dip or wash.

Silvestrini, D. A., Anderson, G. W. and Snyder, E. S., 1958, Chlortetracycline as related to the microbiology and preservation of poultry meat. *Poultry Sci.* **37**, 179.

Use of 10 ppm chlortetracycline in the dip solution during processing delayed the onset of bacterial spoilage in poultry meat about 25 days. Feeding of 1000 gm of the antibiotic per ton of feed to broilers for three, two and one day before slaughter delayed bacterial spoilage approximately 18, 14 and 11 days, respectively. Addition of 500 gm chlortetracycline per ton to the drinking water for one day before slaughter prolonged the keeping quality approximately 11 days. The route of bacterial spoilage in poultry meat appears to start in the visceral cavity tissue and spreads through the muscle tissue to the skin. Production of ammonia-nitrogen from the degradation of tissue protein, increase in bacterial populations and amount of bacterial fluorescence were closely associated with the detection of spoilage by organoleptic examination.

Wells, F. E., Spencer, J. V. and Stadelman, W. J., 1958, Effect of packaging materials and techniques on shelf life of fresh poultry meat. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 425.

Cellophane sheets (300 LSAT), vinylidene chloride copolymer sheets (unevacuated), and vinylidene chloride copolymer bags (partially evacuated to a vacuum of 10 inches) as packaging were studied in control chicken halves and chicken halves treated with 30-ppm solutions of oxytetracycline and of chlortetracycline. Determination of time to spoilage was determined organoleptically and slime smears were made to observe changes in the microflora. Partial evacuation of the containers accounted for increases in shelf-life regardless of the treatment the meat received prior to packaging. Oxytetracycline and chlortetracycline used at 30 ppm were equal in effectiveness.

D3. FISH AND SEA FOOD

Tarr, H. L. A. and Deas, C. P., 1948, Action and sulfa compounds, antibiotics, and nitrite on growth of bacteria in fish flesh. *J. Fisheries Research Board, Can.* **7**, 221.

Bacteriostats were mixed with comminuted fish flesh and stored at 0°C. Neither penicillin nor streptomycin was effective in decreasing the rate of bacterial increase.

Tarr, H. L. A., Southcott, B. A. and Bissett, H. M., 1950, Effect of several antibiotics and food preservatives in retarding bacterial spoilage of fish. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **83**, 35.

Minced flesh of halibut, red spring salmon and brill, were treated with antibiotics and stored at 33° and 37°C for periods up to 10 days. Chlortetracycline, oxytetracycline and chloramphenicol were by far the most effective; quantities as low as 10–25 ppm almost completely inhibited bacterial growth. Subtilin, penicillin and streptomycin had little preservative action.

Partmann, W., 1952, The efficacy of bactericidal ice in preservation of sea fish (in German). *Z. Lebensm.-Untersuch. u.-Forsch.* **94**, 246.

A review of all types of chemical additives to ice (including antibiotics) which may be used for storage of fresh fish. 148 references.

Tarr, H. L. A., Southcott, B. A. and Bissett, H. M., 1952, Experimental preservation of flesh foods with antibiotics. *Food Technol.* **6**, 363.

Chlortetracycline, oxytetracycline and chloramphenicol in the order named proved the most effective inhibitors of growth of the natural mixed bacterial flora of fish and meat at temperatures between 0–21°C, while rimocidin inhibited yeast growth. Others used were streptomycin, penicillin, subtilin, polymyxin B, circulin, neomycin, bacitracin, gramicidin, methylol gramicidin, tyrothricin and an unnamed antibiotic.

Boyd, J., Brumwell, C. and Tarr, H. L. A., 1953, Aureomycin in experimental fish preservation. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **96**, 25.

Experiments were carried out using flaked ice containing 1, 2 and 4 ppm of chlortetracycline. Beheaded and dressed lingcod were iced with ordinary ice and antibiotic ice. After 14 days, fish in ordinary ice had 190 million bacteria per gram and in antibiotic ice (one ppm) 20 million per gram. In another experiment small dressed red spring salmon were immersed in sea water containing 2 ppm chlortetracycline, held at 30°F for 6 days and then iced. These fish had no bacteria, as compared to 25 million per gram on fish stored in ordinary ice for 6 days. Another experiment was carried out dipping coho salmon for one minute in 5 and 10 ppm chlortetracycline and then icing. Results indicate that the original solutions were too dilute for preservation since they were further diluted by the melting ice. However, 50 or 100 ppm chlortetracycline proved very effective.

Boyd, J. W. and Tarr, H. L. A., 1954, Inhibition of mould development in fish products. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **99**, 22.

Into dairy salt the following substances were incorporated by grinding in the concentrations shown: sorbic acid (0.05, 0.1 and 0.2%), sodium benzoate (0.05 or 0.1%) plus sodium dihydrogen phosphate (1%), sodium propionate (0.1%), chlortetracycline (0.01 or 0.02%), or tetracycline (0.01 or 0.02%). Using salt as a control, portions of fresh lingcod were salted by a standard dry-salting procedure. The samples were stored for five days at 40°F to allow sufficient penetration, and then they were sprayed with a suspension of the mold (*Sporendonema epizoum*) which causes "dun" of salted fish, or with a mixture of the red bacteria isolated from a typical sample of "red" discolored salted fish. None of the substances caused any important decrease in the rate at which red bacterial discoloration developed. "Dun" development by mold was effectively inhibited only by sorbic acid.

Farber, L., 1954, Antibiotics as aids in fish preservation. I. *Food Technol.* **8**, 503.

Chlortetracycline, oxytetracycline and neomycin (all at 2 ppm) were tried as preservatives for lingcod, sole, black cod and sablefish fillets and shrimp. Chlortetracycline and oxytetracycline were effective, but neomycin was not.

Gillespie, D. C., Bissett, H. M., Boyd, J. W. and Tarr, H. L. A., 1954, A method of facilitating distribution of germicidal substances throughout ice blocks. *Fisheries Research Board, Can., Progress Repts. Pacific Stas.* **99**, 18.

A relatively uniform distribution of chlortetracycline hydrochloride (1 ppm), but not of sodium nitrite (1000 ppm), was produced throughout 300-lb blocks of ice when carrageen (0.1%) and a small amount of potassium chloride were dissolved in the solution prior to freezing it.

Gillespie, D. C., Bissett, H. M., Boyd, J. W. and Tarr, H. L. A., 1954, Aureomycin in experimental fish preservation. II. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **100**, 12.

Crushed block ice with chlortetracycline (1 ppm) was used to re-ice, after cleaning and washing, previously ungutted sockeye salmon which were in good condition having been packed for 1 to 3 days in ordinary ice. After 9 days the 5 fish packed in ordinary ice had 19 million bacteria per gram while the 5 fish in chlortetracycline ice contained less than 0.26 million bacteria per gram. After 14 days the bacterial counts were 242 and 2.1 million, respectively. It appears that even fish which are not strictly fresh when eviscerated will keep much better in chlortetracycline ice than in ordinary ice.

Four treatments (packing of dressed salmon in ordinary crushed ice, in crushed ice containing 1 ppm chlortetracycline, circulating sea water at 28°F, and circulating ice water at 28°F with chlortetracycline at a level of 2 ppm) were given to fish in a trolling boat for 8 days after catching. Accelerated spoilage tests were carried out by incubating steaks for one day at 50°F. Both the antibiotic ice and circulating sea water with antibiotic caused an extremely pronounced retardation in the rate of spoilage.

Tarr, H. L. A., 1954, Microbiological deterioration of fish post mortem, its detection, and control. *Bacteriol. Rev.* **18**, 1.

Discusses the biochemical activities of bacteria in spoiling of fish, different chemical agents and their effect on these bacteria and various tests for detecting bacterial spoilage.

The only significant antibiotics for the preservation of fish are chlortetracycline, oxytetracycline and chloramphenicol for their bacteriostatic action. Rimocidin inhibited yeast growth.

Tarr, H. L. A., Boyd, J. W. and Bissett, H. M., 1954, Experimental preservation of fish and beef with antibiotics. *J. Agr. Food Chem.* **2**, 372.

Spoilage of whole eviscerated fish was retarded markedly by ices containing 1 to 4 ppm of chlortetracycline, by holding 6 days at -1°C in sea water containing 2 ppm, or by one minute immersion in solutions containing 50 or 100 ppm of the antibiotic prior to icing. In ground beef and fish chlortetracycline suppressed bacterial development but permitted yeast growth, and thiolutin (10 ppm) did not inhibit yeast development in the presence of chlortetracycline to any important extent. Puromycin was devoid of antibacterial activity.

Boyd, J. W., Bissett, H. M. and Tarr, H. L. A., 1955, Effect of addition of Aureomycin to sea water on the viable bacteria present in the viscera of living fish. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* 102, 4.

Live rock cod were held for 24 hours in aerated sea water containing 0, 1 and 5 ppm chlortetracycline. Bacterial counts showed that the antibiotic had at most only a limited bactericidal effect on the viscera of living fish; 1.2 ppm chlortetracycline was detected in the visceral contents of live fish stored in a 5-ppm solution.

Boyd, J. W., Bissett, H. M. and Tarr, H. L. A., 1955, Further observations on the distribution of chlortetracycline throughout ice blocks. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* 102, 14.

Earlier study showed that addition of carrageen (dissolved by heat) to the water used in making ice containing 1 ppm chlortetracycline caused the antibiotic to be fairly uniformly distributed throughout ice blocks. Further work showed that solution is obtained by prior mixing of the dry carrageen with propylene glycol. Carboxymethyl cellulose is also effective in distributing the antibiotic.

Castro, J. O. de, 1955, Antibiotics in fish preservation (in Portuguese). *Conservas de Peixe* 10, 15.

Review article, principally concerned with the experiments of H. L. A. Tarr.

Gillespie, D. C., Boyd, J. W., Bissett, H. M. and Tarr, H. L. A., 1955, Ices containing chlortetracycline in experimental fish preservation. *Food Technol.* 9, 296.

Methods of preparing flake-type and block ice containing added chlortetracycline are described. Relatively uniform distribution of the antibiotic throughout ice blocks is attained by adding carrageen preparations or carboxymethyl cellulose to the water used. Bacterial spoilage of eviscerated fish normally is markedly delayed by icing them with flake or crushed block ice containing about 1 ppm chlortetracycline, rather than ordinary ice, or by holding eviscerated or noneviscerated fish at -2°C in sea water containing 1 to 4 ppm of the antibiotic.

Partmann, W., 1955, Use of bactericidal compounds in dips for fresh fish (in German). *Kältetechnik* 7, 270.

Deep-sea fish can economically be eviscerated and cooled immediately. With vessel storage surface slime increases and if washed at arrival in port 80 to 90% of bacteria are lost. Shelf-life of such fish is increased by a dip in sodium nitrite but use of this compound is illegal in many countries. Chlortetracycline dip is as good as sodium nitrite but is more expensive.

Steiner, G. and Tarr, H. L. A., 1955, Transport and storage of fish in refrigerated sea water: II. Bacterial spoilage of blue-back salmon in refrigerated sea water and in ice, with and without added chlortetracycline. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* 104, 7.

At intervals during storage, steaks from the fish were incubated 1 day at 100°F and bacterial counts were made. The order of preference of various

treatments was: (1) refrigerated sea water with chlortetracycline (0.7 to 1.1 ppm); (2) ice containing 1 ppm chlortetracycline; (3) refrigerated sea water; (4) ordinary ice.

Tomiyama, T., Kuroki, S. and Nomura, M., 1955, Keeping quality of mackerel, *Scomber japonicus*, by using Aureomycin (in Japanese). *Bull. Japan. Soc. Sci. Fisheries* **21**, 958.

Chemical tests for volatile base-N, trimethylamine-N, and histamine hydrochloride content showed that a 1-hour dip in a 10-ppm chlortetracycline brine (5%) increased keeping time 1.7 times. A 2-hour dip in a 40-ppm chlortetracycline brine increased keeping time by 1.4 to 2.6 times depending on test used.

Tomiyama, T., Nomura, M. and Kuroki, S., 1955, Effectiveness of Aureomycin on keeping quality of sardines (in Japanese). *Bull. Japan. Soc. Sci. Fisheries* **21**, 262.

Dipping sardines for 30 minutes or longer in a 5% brine containing 10 to 20 ppm chlortetracycline produced a preservative effect as judged by organoleptic or volatile base-N tests. Overnight storage of sardines in cracked ice containing 10 to 20 ppm chlortetracycline followed by storage at 4° to 8°C without ice prolonged storage life by several days as judged organoleptically or by volatile base-N tests.

Boyd, J. W., Bluhm, H. M., Muirhead, C. K. and Tarr, H. L. A., 1956, Use of antibiotics for the preservation of fish and sea foods. *Am. J. Public Health* **46**, 1531.

Chlortetracycline was more effective than amphomycin, etamycin, bryamycin and furan derivatives in tests on growth of bacteria in minced muscles of lingcod, salmon and halibut. Whole gray cod and sole iced with ice containing chlortetracycline (2.5 ppm) for vessel storage with subsequent accelerated storage at 10°C had much lower bacterial counts than controls. Bacterial counts in minced lingcod flesh showed that addition of chlortetracycline did not create favorable conditions for growth of *Cl. botulinum* Type E or *Staphylococcus aureus*.

Boyd, J. W. and Tarr, H. L. A., 1956, Effect of chlortetracycline and storage temperatures on the quality of shucked oysters. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **105**, 12.

Chlortetracycline was added to the aerated rinse tank used to remove loose sand from shucked oysters. Levels of 2 or 4 ppm chlortetracycline had only slight effect on the bacterial counts of oysters stored at 32°, 40° or 50°F. Antibiotic solutions of 10 or 20 ppm were effective.

Crean, P., Tarr, H. L. A. and Barker, R. B., 1956, Control of post-mortem bacterial spoilage of whales with chlortetracycline. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **105**, 6; Also, 1956, *Western Fisheries* **51**, 32.

Three of 5 whales were treated with chlortetracycline by injection into the visceral cavity and/or by enclosure in the explosive head of the harpoon. Free fatty acid content of oil was 0.07 to 0.7% in 2 treated whales and 2.1% in

one untreated whale. Bacterial counts and volatile base determinations from various tissues also showed the superiority of the treated whales.

Fieger, E. A., Bailey, M. E. and Novak, A. F., 1956, Chemical ices for shrimp preservation. *Food Technol.* **10**, 578.

Effectiveness of ices containing various chemicals was determined in delaying loss of quality and onset of spoilage and in prevention of melanosis in ice-stored shrimp. Ices containing chlortetracycline (10 ppm), sodium chloride (20,000 ppm) and calcium chloride (2000 ppm) extended ice storage life of shrimp 2 to 4 days but were of no value in controlling black spot. Commercial ice was as effective as chlortetracycline ice (1 ppm), sodium bisulfite ice (100 ppm), acid ice (pH 5.0), Fran-Kem ice (500 ppm) and tannic acid ice (500 ppm). Sodium bisulfite ices (1000 or 1500 ppm) delayed deterioration and spoilage approximately 2 days and were very effective in preventing melanosis.

Fieger, E. A., Novak, A. F., Bailey, M. E., McConnell, J. N. and Viosca, P., Jr., 1956, Preserving shrimp quality and beauty. *Southern Fisherman* **16**, 21.

Freshly caught shrimp were headed, washed and divided into eight lots. One lot remained in ice and each of the seven remaining lots was dipped for ten minutes in one of the following solutions: fresh water (A); NaHSO₃, 1000 ppm (B); NaHSO₃, 2500 ppm (C); chlortetracycline, 50 ppm (D); NaHSO₃, 1000 ppm plus chlortetracycline, 50 ppm (E); NaHSO₃, 2500 ppm plus chlortetracycline, 50 ppm (F); and citric acid, 1000 ppm plus ascorbic acid, 1000 ppm (G). Tails were drained for two minutes and packed into waxed cartons or aluminum pans, which were iced and stored at 45°F. Packaged tails showed lower bacteria counts than unpackaged tails stored in crushed ice. Melanosis was inhibited for 14 days by C; for seven days by D, F and G; and for three days by A, B and E. Chlortetracycline in D, E and F inhibited bacterial growth for 14 days and retarded off-odor development without affecting general flavor, but did not extend either the prime-quality range or the total storage life. A, B, C and G did not inhibit bacterial growth. Cooking removed the yellow-green color in tails treated with chlortetracycline.

Firman, M. C., Abbey, A., Darken, M. A., Kohler, A. R. and Upham, S. D., 1956, Effect of Aureomycin chlortetracycline on fish freshness. *Food Technol.* **10**, 381.

Freshness of fish—sea bass, weak fish, croaker, butterfish, scrod, porgy, salmon and halibut—was prolonged successfully by using an ice, a dip or a freezing brine containing chlortetracycline. Antibiotic activity which was determined in the raw treated fish ranged from 0.09 to 5.5 ppm, depending on treatment. Microbial counts and organoleptic observations confirmed the effectiveness of chlortetracycline in extending the storage life of freshly caught fish.

Kline, E. F., Abbey, A., Darken, M. A., Firman, M. C., Kohler, A. R., Miller, W. H., Wiley, W. W., Windlan, H. and Upham, S. D., 1956-57, Improved quality of fish fillets as a result of a food grade of chlortetracycline. *Antibiotics Ann.*, 997.

Whole red fish were treated with ices (5 ppm) and dipping solutions (25 ppm) containing chlortetracycline to prolong storage life. In the first experiment treated whole fish, graded at 14 days out of the sea by examination of eyes, gills, color, texture, odor and taste of the cooked fillet were rated superior. Fillets of these fish were frozen into blocks and processed into fish sticks. Chlortetracycline assays of thawed samples before and after cooking showed higher residues in the skin than in the flesh; the highest level of 0.16 microgram chlortetracycline per gram of skin was completely destroyed by frying and reduced to 0.03 microgram by broiling.

A second experiment used higher concentrations (100 ppm) of chlortetracycline and longer times of dipping; all dip samples were stored in ice containing 5 ppm. Organoleptic tests and microbial counts of whole fish and of thawed fillets showed treated fish to be superior. Frying tests reduced the chlortetracycline residue to zero and broiling significantly reduced residues. In fish stored 10 days on the boat in ice containing chlortetracycline, 30% were rated as excellent and 65% as very good, compared to 10% and 45% in control fish, respectively.

Lerke, P. A. and Farber, L., 1956-57, Prolongation of the keeping quality of fish and shellfish by antibiotics. *Antibiotics Ann.*, 966.

Of 19 antibiotics screened for their ability to prolong the storage life of sole fillets at 42°F, the tetracyclines were most active. Chlortetracycline was somewhat more active at lower concentrations, and oxytetracycline and tetracycline were somewhat comparable in activity. The criteria used were: organoleptic judgment, contents of volatile reducing substances, total volatile nitrogen and trimethylamine nitrogen, and total number of viable aerobic bacteria in press juices of fish and shrimp. Round spawning Pacific herring when stored at 50 to 55°F had a superior shelf-life when dipped for 15 minutes in chlortetracycline solution (15 mcg/ml) and stored in chlortetracycline ice containing 5 mcg/ml. Unpeeled Mexican shrimp stored at 42°F showed a shelf-life extension of four days when dipped in a 15-ppm chlortetracycline solution. Results showed that without refrigeration the antibiotics appear to have only limited usefulness, if any at all.

Ravich-Shcherbo, Y. A., 1956, Prospects of the use of antibiotics in the fishing industry (in Russian). *Rybnoe khoziasstvo USSR* 6, 75.

Review article of experiments by H. L. A. Tarr and in Russian laboratories.

Stern, J. A., Liebman, H. L., Grauer, A. D., Kudo, G. and da Costa, A., 1956-57, Comparative studies of the effects of the tetracycline group of antibiotics in the preservation of fish. *Antibiotics Ann.*, 984.

Oxytetracycline, chlortetracycline and tetracycline were tested for effectiveness in inhibiting bacterial spoilage of noneviscerated English sole. The fish were stored in brines containing different concentrations of the antibiotics. Three chemical tests (volatile acids, volatile bases and trimethylamine determinations) were used in conjunction with organoleptic judgments to evaluate the conditions of the fish. When the antibiotics were used on an equal weight of activity basis at 2 and 5 ppm, the data indicated that oxytetracycline was more effective than chlortetracycline or tetracycline as judged by all three tests. There was no significant difference between chlortetracycline and tetracycline. When the antibiotics were compared on an equal molar basis, no

statistically significant differences among the three antibiotics were obtained when the volatile bases or trimethylamine tests were used. However, tetracycline was less effective than oxytetracycline or chlortetracycline when the test was by volatile acids determination.

Stern, J. A., Liebman, H. L., Munkelt, R. E. and Hatherell, B., 1956-57, The potential application of antibiotics in the salmon canning industry. I. Organoleptic evaluations. *Antibiotics Ann.*, 975.

Oxytetracycline at the levels of 5, 10 and 20 ppm in brines at temperatures of 32°, 40° and 50°F was very effective in extending the freshness of sockeye salmon. In general, all concentrations of the antibiotic were of equal effectiveness. Chlortetracycline used in brines at 5 ppm at 32°F, 10 ppm at 40°F and at 20 ppm at 50°F was as effective as oxytetracycline at the same concentrations. Spraying salmon with oxytetracycline solutions of 20 and 100 ppm also extended the freshness of salmon at room temperature storage.

Tarr, H. L. A., 1956, Control of bacterial spoilage of fish with antibiotics, in Natl. Acad. Sci., Natl. Research Council. Proc. 1st Intern. Conf. on Use of Antibiotics in Agriculture, Washington, D. C., Publ. 397, 199.

General review of subject with examples of studies with ices and sea water containing the antibiotic when used to store fish.

Tomiya, T., Kuroki, S., Maeda, D., Hamada, S. and Honda, A., 1956, A study of the effects of Aureomycin-containing sea water and ices upon the storage life of round herring. *Food Technol.* 10, 215.

Remarkable extension of storage life of round herring resulted after treatment either by storage in sea water containing ice and chlortetracycline (10 ppm) on the boat, by storage in ice containing the antibiotic (5 ppm) after landing, or their combination. The combination of storage with the antibiotic in sea water and in the ice prolonged the storage life approximately 90% at 15° to 20°C and at least 40% at -1° to 2°C.

Abbey, A., Kohler, A. R. and Upham, S. D., 1957, Effect of Aureomycin chlortetracycline in the processing and storage of freshly shucked oysters. *Food Technol.* 11, 265.

Concentrations of chlortetracycline (1, 5, 10, 20 and 30 ppm) were tested in the processing of freshly shucked oysters with varying proportions of oysters in the processing solutions as well as varying exposure periods. Fresh oysters (standards) were commercially processed up to the flotation or blowing step. The freshly shucked oysters were treated with the antibiotic, packed in cans and sealed under commercial conditions. Microbial counts and organoleptic tests were made at intervals up to 33 days, depending on test conditions. The pH trends appeared to be less indicative of freshness in this study. Data presented indicate that chlortetracycline would be useful in maintaining the freshness of shucked oysters.

Bernarde, M. A. and Littleford, R. A., 1957, Antibiotic treatment of crab and oyster meats. *Appl. Microbiol.* 5, 368.

Half-pound quantities of Atlantic Blue Crab were dipped for two minutes in solutions (5, 10, 15, 20, 30, 40 and 60 mcg/ml) of oxytetracycline and chlortetracycline, and were stored at 1°C. At intervals, samples were taken to

determine bacterial counts, pH, antibiotic residues and sensory characteristics. Five gallons of shucked oysters in 50-gallon solutions (5 or 15 mcg/ml) of the antibiotics were "blown" (air under pressure to the tanks) for three minutes, spraywashed, packed in pint cans and sealed. In crab meat, decreased bacterial counts were obtained in some instances but shelf-life was not extended. Similarly as with oysters, the total viable aerobic population was reduced, but storage life was not increased.

Bernarde, M. A., 1957-58, Comparison of tap and distilled water antibiotic dip solutions on storage life of fresh crab meat. *Antibiotics Ann.*, 244.

Small extension of edible life was obtained by dip treatment of fresh crab meat using tap water. A comparative evaluation was made of solutions of oxytetracycline, chlortetracycline, polymyxin and oleandomycin in tap and distilled water. The antibiotic effect was the same in both kinds of water.

Pozo Fernandez, R., Moreno Calvo, J. and de la Camara Cumella, F., 1957, Use of antibiotics in the production of ice and preservation of fish (in Spanish). *Rev. frio* 2, 141.

Studies completed in the field are reviewed and a proposed study in fish preservation to be made at the Experimental Cold Center in Madrid is outlined. The influence of refrigeration temperature, optimum time of treatment, sweet water versus salt water, and antibiotic concentration suitable to the conditions and climate in Spain will be studied.

Tawara, T. and Sasano, Y., 1957, Effect of service water on the stability of chlorotetracycline. II (in Japanese). *Bull. Japan. Soc. Sci. Fisheries* 22, 315.

Active chlorine in service water was suggested as the cause for reduction of chlorotetracycline activity. Tests showed that CTC was stable in sea water and that iron and calcium in the service water did not affect the activity of CTC. In other tests, vitamin C, sodium nitrite, sodium sulfite and sodium thiosulfate when added to the service water were more effective in removing the active chlorine than citric acid or tartaric acid.

Tawara, T. and Sasano, Y., 1957, Effect of service water on the stability of chlorotetracycline. I (in Japanese). *Bull. Japan. Soc. Sci. Fisheries* 22, 721.

The activity of chlorotetracycline was reduced in the service water used to make ice. Activity of CTC in service water dropped from 5 ppm to 1.5 ppm in an hour while activity in distilled water remained about the same. Antibiotic activity in service water acidified with citric acid was fairly stable. The presence of free chlorine as well as pH appeared to affect the stability of CTC.

Ciani, G. and Montefredine, A., 1958, Italian contribution to the preservation of fish with antibiotics (in Italian). *Atti delle V. Giornate Veterinarie sui Prodotti della Pesca, Ancona*, July 20-21.

In their experiments oxytetracycline and chlortetracycline were equally effective. Best results were obtained by dipping the fish in a brine solution containing 5 ppm antibiotic and storing in ice with 5 ppm antibiotic. Results were equally good with fresh water and salt water fish. Shelf-life of shellfish

was extended by treatment with antibiotics, but was not as dramatic as with fish. The antibiotic residue in the treated fish was less than 0.5 ppm.

Southcott, B. A., Baker, E. G., Boyd, J. W. and Tarr, H. L. A., 1958, Comparative effectiveness of tetracycline antibiotics for fish preservation. *Food Technol.* **12**, 108; Abstr., 1957, *Food Technol.* **11**, 26.

Chlortetracycline, oxytetracycline and tetracycline were studied as preservatives: in lingcod fillets dipped in 5-ppm solutions and stored at 0°, 3° and 10°C; in eviscerated lingcod stored in antibiotic ice (100, 300 and 5 ppm); and in eviscerated rock cod stored at -1° to 0°C in 3% NaCl solutions containing 1.5 ppm of the antibiotics, aerated and nonaerated. Controls for each group were used and at intervals the effect of treatment was measured by direct and viable bacterial counts and by an arbitrary odor rating. In fillet dipping or fish icing CTC was consistently more effective. In 3% salt solution storage CTC had somewhat greater effectiveness than the other antibiotics; aeration definitely improved organoleptic results for 20 days' storage.

Stern, J. A., Liebman, H. L., Kudo, G., Olsen, R. A., Farber, L. L. and Grennan, M., 1958, The potential application of antibiotics in the salmon canning industry. II. Chemical and bacteriological evaluations. *Food Technol.* **12**, 132; Abstr., 1957, *Food Technol.* **11**, 26.

Sockeye salmon (*Oncorhynchus nerka*) obtained upon arrival at a cannery were stored at 32°, 40° and 50°F in seawater containing 0.5, 10 and 20 ppm of oxytetracycline. Chlortetracycline was also tested at 32°, 40° and 50°F using levels of 5, 10 and 20 ppm, respectively. Other samples were obtained at the fishing grounds aboard a tender and were sprayed immediately with seawater containing 0, 20 or 100 ppm oxytetracycline. These fish were stored dry, at ambient temperatures (60° to 70°F). At intervals samples were graded organoleptically and were canned. The cans were either sharp frozen or heat processed. Organoleptic, chemical and bacteriological determinations were made on these samples. All levels of the antibiotics used in these experiments seemed equally effective, indicating that brine containing 5 ppm of either antibiotic is of sufficient concentration to give the maximum preservative effect. The 100-ppm spray was more effective than the 20-ppm spray. Extension of storage time depended on the temperature, and the chemical and organoleptic examinations substantiated these conclusions. Assays for residual antibiotic activity in the heat-processed cans were negative.

D4. FRUITS AND VEGETABLES

Bonde, R., 1953, The control of bacterial decay of the potato with antibiotics. *Am. Potato J.* **30**, 143.

Dihydrostreptomycin and streptomycin were very effective and oxytetracycline moderately effective in reducing rot caused by the blackleg bacterium (*Erwinia atroseptica*). Antibiotics (10 ppm) were applied in a 30-minute dip to inoculated potato slices or to cut seed pieces. Penicillin, bacitracin, rimocidin and thiolutin were ineffective. Streptomycin did not inhibit decay caused by *Pseudomonas fluorescens* but chlortetracycline was effective. Results indicate that a mixture of antibiotics should therefore be used.

Smith, W. L., Jr., 1953, Antibiotic treatments for the reduction of bacterial soft rot of packaged spinach. A. C. S., Abstr. of Papers, 124th Meet., p. 31A; Also, 1953, *Agr. Research, U. S.* 2, 6; 1952, *Phytopathology* 42, 175.

Streptomycin sulfate (0.1%) and Tween 20 (0.5%) in water was used as a spray on spinach one or 5 days before harvest or as a 5- or 10-minute dip after harvest. Before packaging, spinach was washed in water containing a 1:1000 dilution of soft rot bacteria. Streptomycin-treated spinach evidenced no decay in one day at 70°F and rarely in 2 days, but it was definitely present in 3. Controls developed decay in one day. Crude streptomycin and oxytetracycline were equally as effective at the same concentrations as streptomycin sulfate. Chlortetracycline was slightly less effective. Rinsing in fresh water after treatment to reduce antibiotic residues did not reduce the effectiveness of treatment.

Geron, A., 1954, Use of germicides (antibiotics and sulfamides) in the preservation of grapes by refrigeration (in Italian). *Ind. conserve, Parma* 29, 32.

Fresh grapes were treated with penicillin, streptomycin, sulfaguanidine, phthalyl sulfacetamide and vitamin K and stored at 2°C, 90% relative humidity. Organoleptic characteristics were preserved and even enhanced in grapes treated with penicillin. Resistance to attack by *Botrytis cinerea* was increased. Shelf-life of the treated grapes was extended 2 months beyond controls.

Nagel, C. W. and Vaughn, R. H., 1954, Sterilization of cucumbers for studies on microbial spoilage. *Food Research* 19, 613.

Mild heat (71.1°C; 160°F) in combination with penicillin (1000 units/ml brine) and streptomycin (200 mcg/ml brine) may be used to sterilize cucumbers without significantly altering their texture.

Smith, W. L., Jr. and Hardenburg, R. E., 1954, Antibiotic and other chemical dips reduce discoloration of packaged cole slaw. Abstr., *Phytopathology* 44, 389.

Cole slaw dipped in a 0.1% streptomycin sulfate solution did not discolor for 3 days, in 1% NaHCO₃ for 2 days, and in 1% NaCl for more than one day. Although 0.1% oxytetracycline or chlortetracycline did control decay, severe injury was caused. Only streptomycin controlled bacterial soft rot.

Cox, R. S., 1955, A preliminary report on diseases of lettuce in the Everglades and their control. *Plant Disease Repr.* 39, 421.

Slime head and jelly butt of lettuce are bacterial rots. In Florida, Cox dipped heads momentarily in a combination of streptomycin and oxytetracycline. Concentrations of 50 ppm to 1000 ppm were highly effective against both diseases. Infection in the untreated samples ranged up to 60% while the range in the treated was from 2 to 9%. A 250-ppm solution painted on the butts of lettuce reduced decay from 60% in the controls to approximately 4% in the treated.

Brody, H. D. and Francis, F. J., 1956, The effect of streptomycin sulphate on prepackaged spinach. *Pre-Pack-Age* 10, 29.

Bacterial soft rot in prepackaged spinach was reduced approximately 40% by a one-minute dip in a 0.05% solution of streptomycin sulfate. A residue

of 25 to 40 ppm was found after cooking. A taste panel could not detect a difference in taste or appearance between a cooked sample containing 30 ppm streptomycin and the controls. The antibiotic treatment did not change the vitamin C content of the spinach but reduced the apparent respiration rate.

Cox, R. S., Carroll, V. J. and Benedict, R. A., 1956, Studies on the etiology and control of the radish pit disease. Presented 48th Ann. Meet., Am. Phytopathol. Soc.; Abstr., 1957, *Phytopathology* 47, 7.

Following 5-minute dip treatments in oxytetracycline solutions (10, 20 and 40 ppm) and streptomycin (40 ppm) solution radishes were held in perforated cellophane bags at 10°, 15°, 20°, 25°, 30° and 35°C. After five days, complete control was found with 40 ppm oxytetracycline. Streptomycin was not as effective.

Goodman, R. N. and Johnston, M. R., 1956-57, Stability of streptomycin in apple and potato tissue. *Antibiotics Ann.*, 1006.

A portion of this paper deals with the use of streptomycin in postharvest treatment of potatoes. Streptomycin proved to be very stable in stored potatoes, showing only a minor loss of activity after 4 months of storage.

Koch, G. and Carroll, V. J., 1956-57, Prevention of post harvest decay with antibiotics. *Antibiotics Ann.*, 1010.

Oxytetracycline, neomycin, polymyxin and streptomycin were tested for retarding spoilage of peas, broccoli, lima beans, cauliflower and spinach. Oxytetracycline was far superior to the other antibiotics tested although all showed some extension of storage life. A dip in 25-ppm oxytetracycline solution for $\frac{1}{4}$ to 5 minutes more than doubled the storage life at 30°C and 70 to 85% relative humidity.

Becker, R. F., Goodman, R. N. and Goldberg, H. S., 1957-58, Prolonging the shelf life of refrigerated prepackaged spinach with antibiotics. *Antibiotics Ann.*, 229.

Spinach was dipped for one minute in solutions containing 25 mcg/ml oxytetracycline, chlortetracycline or streptomycin, packed in perforated polyethylene bags and stored at 45°F. In some samples artificial inoculum (*Erwinia carotovora*) was added to the wash water. Less decay was noted in treated spinach when artificial inoculation was used. In samples without any inoculum, decay was slower and the antibiotics had less effect on the amount of decay. Bacterial population counts indicated that the antibiotics suppressed the number of organisms present. With oxytetracycline and chlortetracycline, activity was not detectable after the treated spinach had been cooked. With streptomycin, 2.05 mcg/ml remained in the spinach after cooking.

Carroll, V. J., Benedict, R. A. and Wrenshall, C. L., 1957, Delaying vegetable spoilage with antibiotics. *Food Technol.* 11, 490.

Solutions of oxytetracycline hydrochloride, streptomycin sulfate and polymyxin B sulfate as dip treatments were tested for decay control in a salad mix and in each of the salad components (cucumber, chicory, escarole, iceberg lettuce, radish, red cabbage). The vegetables were stored at several temperatures and observed daily for microbial spoilage. Samples treated with the antibiotics and stored at 5°C demonstrated the delay of decay by use of an

antibiotic combined with refrigeration. Oxytetracycline afforded the greatest protection against vegetable spoilage.

DiMarco, G. R. and Davis, B. H., 1957, Prevention of decay of peaches with post-harvest treatments. *Plant Disease Repr.* **41**, 284.

Several chemicals were tested in 1955 to determine their value in controlling brown rot of harvested peaches caused by *Monilinia fructicola* (Winter) Rehm. The fruit was inoculated, incubated for three hours and dipped for one minute in the chemicals. Nystatin (100 ppm) and sodium *o*-phenylphenate (1000 ppm) gave the best results and were tested again in 1956 in a hydrocooler using uninoculated, field-run peaches of the variety in season. Both materials gave substantial reduction of brown rot and *Rhizopus* rot. Nystatin gave better results in most tests.

Goodman, R. N., Johnston, M. R. and Goldberg, H. S., 1957-58, Residual quantities of antibiotics detected in treated plant tissue. *Antibiotics Ann.*, 236.

Significant residual quantities were found in treated plant tissue. In potatoes, there was 0.362 mcg/ml oxytetracycline four months after treatment with a dip in 100 mcg/ml and tetrachlorethylin at 100 mcg/ml. Bioassays did not show residues of cycloheximide, anisomycin or rimocidin in potatoes. Cabbage (slaw) dipped for 15 seconds in an oxytetracycline solution (25 mcg/ml) showed presence of the antibiotic for 16 days. Dip-treated spinach (1000 mcg/ml streptomycin for one minute) showed the antibiotic to be active after being blanched in live steam for three minutes.

Anonymous, 1958, Post-harvest spoilage. *J. Agr. Food Chem.* **6**, 16.

Postharvest losses prevent 25% of all fruits and vegetables harvested from reaching consumers. Refrigeration is still the most effective tool to protect fresh produce from rapid spoilage. Researchers hope to break the time limitations of refrigeration by use of chemicals or antibiotics, alone or with hydrocooling or refrigeration.

Johnston, M. R., Finley, N. and Edwards, J. D., 1958, Antimicrobial treatments on the stored potato microflora. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 38.

Effects of antimicrobial agents on the microflora of stored prepackaged potatoes were studied. Sorbic acid, tetrachloroethylene, griseofulvin, oxytetracycline, chlorotetracycline and streptomycin were used singularly and in two- and three-way combinations. Potatoes in sealed polyethylene bags were stored at 45°F, and 11 microbiological examinations were made at intervals during eight weeks of storage. Three differential media were used, and a nutrient agar for total count. Data showed that, in addition to the direct effect of an agent on a group of the microflora, usually there is a stimulation of another segment of the microflora. Also, a single nutrient medium for a gross count of the microflora does not give a true indication of the efficacy of an agent.

D5. DAIRY PRODUCTS

Curran, H. R. and Evans, F. R., 1946, The activity of penicillin in relation to bacterial spores and the preservation of milk. *J. Bacteriol.* **52**, 89.

Penicillin is not especially effective against *Bacillus cereus*, *B. mycoides*, *B. albolactis*, *B. netiens* and spores of *Clostridium botulinum*. Therefore it can be concluded that penicillin has no application in food preservation. In combination with mild heating, it might have ability as a spoilage-delaying agent in nonfood materials.

Curran, H. R. and Evans, F. R., 1946, The activity of streptomycin in relation to bacterial spores and the preservation of milk. *J. Bacteriol.* **52**, 142.

Tubes of sterile (autoclaved) milk were seeded with washed bacterial spores (approx. 50,000 per ml), heated at 95°C for 15 minutes, cooled, 5 units per ml of streptomycin added, and stored at 30°C. Streptomycin in ordinary concentration has very limited activity against bacterial spores and would not control *Clostridium botulinum* and Putrefactive Anaerobe 3679 at 100 units per ml.

Katznelson, H. and Hood, E. G., 1949, Influence of penicillin and other antibiotics on lactic streptococci in starter cultures used in Cheddar cheese-making. *J. Dairy Sci.* **32**, 961.

Of six antibiotics used (penicillin, streptomycin, chlortetracycline, chloramphenicol, subtilin and bacitracin at 0.05 and 1.0 ppm) against 45 strains of lactic streptococci from starter cultures, penicillin was the most effective inhibitor of acid production. Subtilin was next best.

Poetschke, G., 1950, Disinfection and preservation of raw milk with streptomycin (in German). *Monatsschr. Kinderheilk.* **98**, 177.

Addition of streptomycin (20 ppm) to raw milk reduced bacterial count and inhibited growth of *Escherichia coli*. The stools of infants fed this milk usually showed presence of *E. coli* (which sometimes disappeared for a short time) and no change in the number of lactobacilli. At the end of a year, the only change was the development of streptomycin resistance in the coli bacilli.

Auclair, J., 1951, Inhibitory action of penicillin on the lactobacilli used in the manufacture of Gruyère cheese (in French). *Lait* **31**, 121.

Penicillin up to 3 units per ml of milk did not substantially inhibit development of acidity of *Lactobacillus helveticus*, but did markedly reduce acid development in *L. lactis* cultures.

Hirsch, A., Grinsted, E., Chapman, H. R. and Mattick, A. T. R., 1951, Inhibition of an anaerobic sporeformer in Swiss-type cheese by a nisin-producing streptococcus. *J. Dairy Research* **18**, 205.

Development of clostridia in Gruyère cheese can be arrested by adding to the usual leavening agent a culture of *Streptococcus lactis* which produces nisin.

Oeklitz, H. W. and Schmidt, E. F., 1951, Preservation of human milk with Aureomycin (in German). *Arch. Kinderheilk.* **142**, 21.

Streptomycin and citric acid were not as effective as chlortetracycline.

Wilkowske, H. H. and Krienke, W. A., 1951, Influence of penicillin in milk on total and coliform bacterial plate counts. *J. Milk and Food Technol.* **14**, 92.

Storage at 10°C for 72 hours of milk containing one unit penicillin per ml resulted in no significant increase in total plate counts; the counts increased considerably in control samples under similar conditions. It is recommended that penicillin not be added to market milk.

Bindewald, H., 1952, Preservation of human milk with streptomycin or citric acid (in German). *Deut. med. Wochschr.* **77**, 1015.

Streptomycin is superior to citric acid for the preservation of mother's milk.

Greene, V. W. and Bell, J. M., 1952, Preserving raw milk with various antibiotic preparations. *Sci. Agr.* **32**, 619.

Chlortetracycline and oxytetracycline added to raw milk in the order of 10 ppm effectively inhibited both acid production and bacterial growth for 20 hours at 37°C.

Inomoto, Y. and Hashida, W., 1952, Application of several antibiotics to the food industry. I. Preservation of cow milk (in Japanese). *J. Fermentation Technol., Japan* **30**, 287.

Acid production and coagulation of milk was checked by chloramphenicol, chlortetracycline and oxytetracycline. The above antibiotics and also patulin were effective against putrefying microorganisms isolated from milk and dairy products for short preservations.

Kooy, J. S. and Pette, J. W., 1952, Inhibition of the growth of lactate-fermenting butyric acid bacteria by antibiotics from lactic acid streptococci (in Dutch). *Neth. Milk Dairy J.* **6** (4), 302; Abstr., 1953, *J. Dairy Sci.* **36**, A52.

Eleven of 350 strains of lactic acid streptococci produced antibiotic substances against other lactic acid streptococci. All the antibiotic producers were classified as *S. lactis*.

Kooy, J. S. and Pette, J. W., 1952, The inhibition of butyric acid fermentation in cheese by using antibiotic producing streptococci as starter (in Dutch). *Neth. Milk Dairy J.* **6** (4), 317; Abstr., 1953, *J. Dairy Sci.* **36**, A52.

When an antibiotic producing strain of *S. lactis* was added to milk inoculated with *Cl. tyrobutyricum*, butyric acid fermentation was inhibited. In one case the butyric acid bacteria did grow, due to the rapid destruction of the antibiotic by *L. plantarum*.

Kooy, J. S., 1952, Strains of *Lactobacillus plantarum* which inhibit the activity of the antibiotics produced by *Streptococcus lactis* (in Dutch). *Neth. Milk Dairy J.* **6** (4), 323; Abstr., 1953, *J. Dairy Sci.* **36**, A53.

The antibiotics produced by strains of *S. lactis* were destroyed by certain strains of *L. plantarum*. In cultures containing mixed strains of *L. plantarum*, the destruction of antibiotics could be prevented by adding strains of *L. casei*.

McClintock, M., Serres, L., Marzolf, J. J., Hirsch, A. and Moequot, G., 1952, Inhibitory action of nisin-producing streptococci on the development of anaerobic sporeformers in processed Gruyère cheese (in French). *J. Dairy Research* **19**, 187.

Nisin (50–100 units per gram of cheese) was added in the making of processed Gruyère cheese at the time of melting by adding a milk culture of nisin-producing streptococci. The proliferation of Clostridia was markedly reduced.

Berridge, N. J. and Mattick, A. T. R., 1953, Some applications of antibiotics to dairying. *Intern. Dairy Congr., Proc. 13th Congr.* **3**, 1104.

Nisin, or streptococcus starters producing nisin, can be used to control the blowing of raw and processed cheese. Lactenin is a bacteriostatic mixture of substances occurring in fresh milk; partial separation has been achieved.

Berridge, N. J., 1953, The antibiotic nisin and its use in the making and processing of cheese. *Chem. & Ind., London*, 1158.

Nisin is known to occur naturally although not frequently in normal milk. Gassy fermentations in raw cheese made with milk inoculated with clostridia have been controlled by using nisin-producing starter cultures. Gassy fermentation in processed cheese was greatly retarded by 100 units of nisin which prevented blowing in 95% of the samples for 60 days while all the controls had blown within 15 days. A culture of nisin-producing streptococcus in milk is being used with success in the commercial manufacture of processed cheese in France.

Hashida, W., 1953, Applications of several antibiotics to the food industry. II. Applications of several antibiotics to cow milk (in Japanese). *J. Fermentation Technol., Japan* 31, 15.

Antibiotics added to cow's milk within 4 hours of milking gave the following preservation at 30°C: streptomycin and penicillin (200 ppm), 1 day; chloramphenicol (100 ppm), 2 days; patulin (200 ppm), 3 days; chlortetracycline and oxytetracycline (100 ppm), 4 days.

Hashida, W. and Asai, T., 1953, Applications of several antibiotics to the food industry. III. Application to the preservation of cow milk (in Japanese). *J. Fermentation Technol., Japan* 31, 112.

Mixtures of antibiotics added to raw cow's milk and the time of preservation are as follows: patulin (100 ppm) plus chlortetracycline (100 ppm), 10 days; penicillin (100 ppm) plus chlortetracycline (100 ppm), 8 days; and penicillin plus chloramphenicol, patulin plus chlortetracycline and chlortetracycline plus penicillin (each at 20 ppm), 3 days. Combination of chloramphenicol and chlortetracycline or patulin and chlortetracycline were not very effective.

Shiveler, G. and Weiser, H., 1953, The effect of selected antibiotics upon the survival of microorganisms in raw and pasteurized milks. *J. Milk and Food Technol.* 16, 125.

Penicillin (3 ppm), dihydrostreptomycin (10 ppm) and chlortetracycline (10 ppm) effectively inhibited bacterial multiplication for 24 hours in raw milk and for 48 hours in pasteurized milk. At the levels used, chlortetracycline was the most effective.

Hirsch, A. and Grinsted, E., 1954, Methods for cultivation and counting of anaerobic sporeformers from cheese with observations on the action of nisin. *J. Dairy Research* 21, 110.

Concerned with influence of nisin on clostridia.

Shahani, K. M., Gould, I. A., Weiser, H. H. and Slatter, W. L., 1954-1955, Effect of heat treatments on streptomycin and chlortetracycline in milk. *Antibiotics Ann.*, 353.

Dihydrostreptomycin and streptomycin (1.0 to 4.14 ppm) was inoculated into fresh raw milk and pasteurized at 143°F for 30 minutes. An average of 17.6% of the antibiotic activity was lost. Dihydrostreptomycin was less heat-

labile than streptomycin sulfate. During storage of raw milk inoculated with a streptomycin sulfate 18.0, 9.4 and 7.7% potency was lost during the first, second and third weeks, respectively. When similar samples were pasteurized, 14.8% activity was lost due to pasteurization and 18.6, 6.9, 4.6 and 3.2% during the first, second, third and fourth weeks, respectively. The streptomycin was completely inactivated when autoclaved at 15 lb pressure for 15 minutes. If the milk was sterilized before adding streptomycin there was no loss of activity during storage for 4 weeks. It appears that microorganisms, enzymes and possibly some other milk constituents in raw milk contribute to antibiotic inactivation. Certain physical and chemical changes occurring during autoclaving may be responsible for the stability of streptomycin in sterile milk.

Some work was done with chlortetracycline, and it was observed that the antibiotic is more heat-labile in water than in milk or phosphate solution. When milk containing penicillin, streptomycin or chlortetracycline was pasteurized, 10 to 17% antibiotic activity was lost.

Shahani, K. M., Gould, I. A., Weiser, H. H. and Slatter, W. L., 1954, Effect of heat treatments on antibiotic content of milk. *Abstr., J. Dairy Sci.* **37**, 647.

Milk containing 0.15 to 1.26 units penicillin per ml lost 0.0 to 15.0% of the antibiotic activity when pasteurized at 143°F for 30 minutes and 4.0 to 16.0% at 160°F for 30 minutes; 16.7% more activity was lost upon storing the milk for 7 days at 0°-2°C. Forty-five to 69.0% penicillin was inactivated when milk was autoclaved at 15 lb pressure for 15 minutes. Seven days later 9.9% more activity had been lost.

Stoltz, E. I. and Hankinson, D. J., 1952, The effects of antibiotics in milk on human intestinal coliform bacteria. *Bacteriol. Proc.* **52**, 60; Also, 1954, *J. Milk Food and Technol.* **17**, 76.

Each subject was given milk containing either penicillin, streptomycin or chlortetracycline at levels representative of residuals of these antibiotics found in market milk supplies. Penicillin (0.1 and 1.0 units per ml) did not reduce number of coliform bacteria; however chlortetracycline (0.25 and 0.5 ppm) and streptomycin (0.5 and 1.0 mg per ml) reduced the number greatly.

Torre, G. D., 1954, Quantitative relations between the normal microbial flora of raw milk and certain antibiotics (in Italian). *Latte* **28**, 221.

Angelotti, R., Weiser, H. H., Slatter, L. L. and Gould, I. A., 1955, The effect of antibiotics upon the microflora of milk. *Appl. Microbiol.* **3**, 234.

Screening tests showed chloramphenicol, chlortetracycline and oxytetracycline to be effective against a wide range of bacterial types occurring in milk. Bacitracin and penicillin had narrow antibacterial spectra. Oxytetracycline and chlortetracycline caused an immediate reduction in numbers of organisms followed by an inhibition of growth and acid production.

Berridge, N. J., 1955, Inhibitory substances of bacterial and other origins in milk and milk products. *J. Sci. Food Agr.* **6**, 65.

Review with 31 references. Approximately 10% of 2647 cultures of lactobacilli tested showed inhibitory activity. The inhibition was shown to be due to hydrogen peroxide produced by nonmultiplying cells. Inhibitors produced by streptococci and other inhibitors occurring in raw milk are discussed.

Romanovich, T. G., 1955, Utilization of antibiotics in the dairy industry (in Russian). *Moscow Vsesoiuzn. Nauch.-Issled. Inst. Moloch. Promysh. Sborn. Ref. Nauch. Rabot* **1**, 45.

Bastoni, G., 1956, Antibiotics as milk preservatives (in Italian). *Latte* **30**, 888.
Short report is given of the extension of shelf-life for 240 hours of pasteurized milk.

Elliott, L. E. and Romoser, G. L., 1956, Studies on recovery of antibiotic activity from egg albumen. Presented 45th Ann. Meet., Poultry Sci. Assoc.; Abstr., 1956, *Poultry Sci.* **35**, 1141.

In the assay of eggs for antibiotics, masked activity is revealed when the specimen is diluted. Results showed that the phenomenon may be partially caused by a chemical binding of the antibiotic by the albumen and that the mechanism may be related to the agar diffusibility of the albumen as an antibiotic carrier. Data are presented of the relative stability of the tetracyclines in egg albumen stored at various temperatures.

Laurenza, A. and Pianese, G., 1956, Effects of antibiotics on the bacterial flora of milk (in Italian). *Giorn. batteriol. e immunol.* **49**, 297.

Penicillin, 0.001 to 10 I. U. per ml, and streptomycin, 0.01 to 5 mcg/ml, each inhibited the development of bacteria in milk, and together were more effective than either alone. The effects lasted 24 hours in milk kept at 22°C and at least 48 hours in samples in an icebox.

Miller, W. A., 1956, Research notes. The effect of coating the shells of washed eggs, that formerly were dirty, with antibiotics, upon subsequent spoilage. *Poultry Sci.* **35**, 241.

Six cases of dirty current receipt eggs were washed in a household detergent; the eggs from each case were coated with a water solution of a different antibiotic, fan dried, but not oiled, and placed in cold storage. Subsequent examination for egg-spoilage bacteria showed no detectable effect of the antibiotics upon the number of bacteria in the eggs or the number of eggs undergoing spoilage when compared with controls.

Shahani, K. M., Gould, I. A., Weiser, H. H. and Slatter, W. L., 1956, Observations on antibiotics in a market milk supply and the effect of certain antibiotics on the keeping quality of milk. *Antibiotics & Chemotherapy* **6**, 544.

Penicillin and streptomycin at either 1 or 25 ppm were ineffective in retarding spoilage of raw milk. One ppm of either chlortetracycline or oxytetracycline had a slight effect but at 25 ppm they extended the storage life of raw milk several days. One ppm of penicillin or streptomycin in combination with pasteurization improved the keeping quality of milk by about 4 days, and 25 ppm by about 7 to 8 days. Chlortetracycline and oxytetracycline were more effective than penicillin and streptomycin, and delayed the microbial spoilage of pasteurized milk by about 2 to 3 weeks.

Shahani, K. M., Gould, I. A., Weiser, H. H. and Slatter, W. L., 1956, Stability of small concentrations of penicillin in milk as affected by heat treatment and storage. *J. Dairy Sci.* **39**, 971.

Effects of heat and storage on the loss of potency of small concentrations (0.13 to 1.07 I. U. per ml) of K penicillin in milk, 1% phosphate buffer (pH 6.0), and water were studied. The heat stability also was compared to five different penicillins added to milk and found to vary. With heat, K penicillin was destroyed in an increasing order in milk, buffer and water. On storage, penicillin lost its potency at a faster rate in milk and water than in buffer. Also, storage losses were less in milk heated at 160° and 250°F than in the raw milk or samples pasteurized at 143°F. Within the limits studied, concentration of the antibiotic and degree of inactivation by heating or storage exhibited no relationship. Penicillin in milk was more heat stable, but less stable during storage, than streptomycin and chlortetracycline in previous studies.

Whitehead, H. R. and Lane, D. J., 1956, The influence of penicillin on the manufacture and ripening of cheddar cheese. *J. Dairy Research* 23, 355.

The addition of penicillin to cheese milk (above 0.10 unit/ml) delayed acid production by starter in the cheese curd; any effect on cheese quality was caused by the delay in acid production and to a high final pH in the cheese. Penicillin did not appear to have a direct effect on the ripening process.

Elliott, L. E. and Romoser, G. L., 1957, Studies on the effect of a chlortetracycline treatment on the green-rot spoilage of eggs. Presented 17th Ann. Meet., Inst. Food Technologists; Abstr., 1957, *Food Technol.* 11, 18.

Preliminary study showed that at least 80% of added antibiotic activity was reclaimed when albumen containing antibiotics of the tetracycline group was diluted 1:3. Paired eggs of known porosity were selected; one egg was evacuated, the contents replaced with an equal volume of sterile saline and sealed. Each pair was immersed for 10 minutes in dip solutions of varying chlortetracycline concentration. Assays showed that up to 50% more antibiotic was assayed in the saline than in the albumen. Also, substantial portions of antibiotic were recovered in the various components of the egg, forecasting the possibilities of an antibiotic dip to prevent bacterial spoilage of eggs. Preliminary results are reported of incidence of green-rot spoilage in market eggs dipped in chlortetracycline solutions.

Galesloot, Th. E., 1957, Effect of nisin on the growth of bacteria connected or possibly connected with bacterial processes in cheese and processed cheese (in Dutch). *Neth. Milk Dairy J.* 11, 58.

The advantages and disadvantages of nisin-producing starters for cheese making were studied. A sterile nisin filtrate prepared from a clotted milk culture or a sterile filtrate made of a 10% water suspension of a commercial preparation of nisin was added to bacteria cultures of summer or winter milk at a concentration of 80 Reading units nisin per milliliter. This concentration inhibited strongly the growth of many strains of bacteria concerned or possibly concerned with bacterial processes in cheese and processed cheese. It is concluded that addition of nisin increases the keeping quality of processed cheese.

Galesloot, Th. E., 1957, Nisin production by antibiotic starters (in Dutch). *Neth. Milk Dairy J.* 11, 74.

The nisin production of three starters, which were transferred daily, decreased after several months, sometimes rather suddenly. This decrease

occurred also if the starters were used in cheese making. Plating out of these starters on agar showed that many colonies did not produce nisin while others had a normal production, i.e., nonnisin-producing variants had arisen. Suitable nisin-producing starters were recognized among the old ones by the method of Chevallier. When a dairy uses an antibiotic starter, it must be checked periodically for the presence of nonnisin-producing variants. It is advisable to obtain periodically a new starter at the optimum level.

Hawley, H. B., 1957, Nisin in food technology—1. *Food Manuf.* **32**, 370.

Nisin is an inhibitory metabolite produced by a cheese-starter organism and is the first antibiotic substance to be accepted by many countries for the control of certain types of bacterial spoilage. A comprehensive review is presented of its discovery and properties, and its present use in natural and processed cheeses.

Hiscox, E. R. and Briggs, C. A. E., 1957, Reviews of the progress of dairy science. Section B: Bacteriology and mycology applied to dairying. *J. Dairy Research* **24**, 387.

Present status is given of the antibiotics, nisin and penicillin, in cheese starters and in cheese production.

Rosell, J. M. and Matallana, S., 1957, Results of nisin culture producers in the prevention of butyric fermentation in cheesemaking (in Spanish). *Rev. espan. leche* **23**, 25.

Nisin is nontoxic and is effective against anaerobic organisms, especially *Clostridium tyrobutyricum*. In England the compound is used commercially in cheesemaking and also in canned foods, sausages, breadmaking. In this study nisin was used in 390 batches of cheese made with milk heavily contaminated with *Cl. tyrobutyricum*. Butyric fermentation in cheese appears 10 to 15 days after making, but can appear after 2 to 3 days, especially in cheeses which do not have the necessary acidity (pH 4.9 to 5.0) in the molds. Use of nisin completely controlled the fermentation in the batches studied. A new nisin culture (1%) was used every 10 to 15 days because cultures became contaminated in the factory. The nisin was added during mixing when the curd was finished and ready to be molded.

Shahani, K. M., 1957, The effect of heat and storage on the stability of Aureomycin in milk, buffer, and water. *J. Dairy Sci.* **40**, 289.

Stability of low concentrations (0.30 to 0.62 mcg/ml) of chlortetracycline in milk, phosphate buffer and water were determined by the disk assay method with *Bacillus cereus* as the test organism. Heating inactivated the antibiotic in increasing order in milk, buffer and water. This was a first-order reaction at 145° and 160°F in milk and buffer, but not in water at 160°F. Activity was lost during storage of five weeks, but the rate was slower in the heated than in the unheated samples. Heating at 143° or 160°F for 30 minutes, with or without prolonged storage, did not completely inactivate the antibiotic in milk, buffer or water.

D6. YEAST FERMENTATION

Dal-Cin, G., 1948, Antibiotics in wine making. *Riv. viticolt. e enol., Conegliano* **1**, 335.

The addition of 0.015–0.025% Biamicina (an Italian antibiotic) was found to regulate fermentation.

Day, W. H., Serjak, W. C., Stratton, J. R. and Stone, L., 1953, Antibiotics as contamination-control agents in grain alcohol fermentations. A. C. S., Abstr. of Papers, 124th Meet., p. 23A.

Bacitracin and streptomycin failed to inhibit the bacteria. The following antibiotics inhibited bacterial development without affecting yeast growth or fermentation: tyrothricin (300 to 500 ppm); oxytetracycline (10 to 20 ppm); chlortetracycline (18 to 50 ppm); chloramphenicol (7 to 20 ppm); and penicillin (0.75 to 2.0 units per ml).

Strandskov, F. B. and Bockelmann, J. B., 1953, Antibiotics as inhibitors of microbiological contamination in beer. *J. Agr. Food Chem.* **1**, 1219.

Several antibiotics were studied for their effect on bacteria and secondary yeast contamination in the brewery. A concentration of 0.005 units per ml of polymyxin B was found to be the most effective in controlling the gram-negative bacterial infection of yeast. Addition of bactericidal concentrations of the antibiotics studied actually stimulated growth. This is believed to be due to elimination of bacteria, not to any direct effect upon the yeast. Penicillin was found to be most effective in controlling gram-positive lactic acid bacteria in finished beer. Thiolutin plus polymyxin B or penicillin (5 ppm each) inhibited the growth of secondary yeast.

Perez-Salas y Lamo de Espinosa, J., 1954, Antiseptics for the preservation of wines (in Spanish). *Semana vitivinic.* **9** (405), 2.

Ribéreau-Gayon, J., 1954, The future of antibiotics in enology (in French). *Monit. vinic.* **99** (8), 3.

Saenz de Valluerca Perea, F., 1954, Antiseptics and antibiotics in enology (in Spanish). *Semana vitivinic.* **9** (399), 2.

Schanderl, H., 1954, On the use of antibiotics in wine (in German). *Wein.-Wiss. Beih. Fachz. deut. Weinbau* **9**, 340.

Visor, F. C. and Prescott, F. J., 1954, Antibiotics in the brewing industry. *Brewers Dig.* **29**, 49T.

When 0.05 ppm polymyxin B is added to pitching yeast, complete control is obtained over the normal gram-negative contaminating flora. The polymyxin B does not appear in the final beer since it is selectively absorbed on commercial filter aids. The antibiotic need be added only once every 4 to 9 fermentations.

Strohm-Hoffmann, U., 1956, Use of preservatives in the brewery (in German). *Brauwissenschaft* **9**, 254.

Methods to improve the biological stability of beer, such as pasteurization, ultraviolet rays, x-rays, gamma rays, cathode rays and beta rays, produce an undesirable effect on the taste. Use of antioxidants, antiseptics and antibiotics has given better results. The use of the antioxidant, ascorbic acid, is generally permitted in France and most of Germany. Numerous reports show experience with antiseptics and antibiotics. Polymyxin has proved to be especially effective when added to the pitching yeast at a concentration of 10 mg per

hectoliter of wort. However, approval of the use of antibiotics and antiseptics must be obtained after extensive tests to show the safety of the compounds.

E. PROCESSED FOOD POSSIBILITIES

Godkin, W. J. and Cathcart, W. H., 1952, Effect of antibiotics in retarding the growth of *Micrococcus pyogenes* var. *aureus* in custard fillings. *Food Technol.* **6**, 224.

Experiments with autoclaved fillings showed that 0.6 ppm to 1.0 ppm of chlortetracycline and oxytetracycline were effective against *Micrococcus pyogenes* during a 24-hour incubation period at 37°C. Penicillin showed slight effectiveness at 1.2 ppm. Bacitracin (40–60 ppm) and chloramphenicol (4–5 ppm) were valueless. Subtilin demonstrated inhibitory action at 10 ppm and bactericidal action at 20 ppm.

With nonautoclaved fillings 1.0 ppm of chlortetracycline and oxytetracycline were effective against *M. pyogenes*. Neither of these antibiotics demonstrated control against heat-resistant contaminating bacilli and the fillings spoiled readily.

Godkin, W. J. and Cathcart, W. H., 1953, The complementary action of subtilin and Terramycin in preserving custard fillings. *Food Technol.* **7**, 282.

One hundred ppm of 70% potency subtilin and 10 ppm oxytetracycline in a custard filling will retard the growth of food poisoning strains of enterococci, salmonella, micrococci and the normal heat-resistant spoilage organisms for a period of 3 days at summer temperature.

Editorial, 1956, Antibiotics proving value as food freezing ally. *Quick Frozen Foods* **18**, 59.

Editorial review of antibiotics in food preservation with additional comments on their use in frozen foods.

Ordal, Z. J. and Brown, W. L., 1956–57, The effect of oxytetracycline on the keeping quality of cured hams. *Antibiotics Ann.*, 860.

The addition of oxytetracycline (15 or 45 mcg/ml) to the curing brine of low sodium chloride content (4%) markedly extended the storage life of hams. The antibiotic retained its activity during curing but a high percentage was lost during smoking. During storage at 85°F, the remaining oxytetracycline activity gradually decreased, but was detectable after 23 days of storage. Under commercial conditions, cured hams are generally stored at 34° to 40°F. Such hams would develop a different microbial flora, and additional experiments are necessary to determine whether oxytetracycline would have a comparable effect.

Hawley, H. B., 1957, Nisin in food technology—1. *Food Manuf.* **32**, 370.

Nisin is an inhibitory metabolite produced by a cheese-starter organism and is the first antibiotic substance to be accepted by many countries for the control of certain types of bacterial spoilage. A comprehensive review is presented of its discovery and properties, and its present use in natural and processed cheeses. Potential applications in canned goods—vegetables, tomato products, fruits, meat and meat products, fish and crustaceans, evaporated milk and milk products—are discussed.

Hawley, H. B., 1957, Nisin in food technology—2. *Food Manuf.* 32, 430.

The possible uses are described of nisin in the preservation of semipreserved meats, open-pack meats, sausages, pickles, beverages (sterilized and chocolate milks), and cream and butter. Use of nisin also is discussed in combating the dangers of food poisoning.

Uusimaki, P., 1958, Treatment of cucumbers with oxytetracycline. *Food Trade Rev.* 28, 37.

Use of oxytetracycline reduced spoilage of delicacy and salt cucumbers to 10% of that experienced previously. Ingredients dipped in a solution of oxytetracycline (10 to 25 ppm) were processed according to the regular preservative formula, using short-time pasteurization and addition of oxytetracycline at 7 ppm of the total mixture.

F. ANTIBIOTIC RESIDUES

Kersey, R. C., Visor, F. C. and Wrenshall, C. L., 1953–54, Residual antibiotic levels in food products during storage and processing. *Antibiotic Ann.*, 438.

A study of residual levels of antibiotics used in foods during processing and storage.

To a test tube containing 10 ppm oxytetracycline and 10 grams of peas in 1% glucose, a million spores of *Clostridium sporogenes* (ATCC 7953) was added. The sealed tubes were subjected to 100°C for varying lengths of time. Tests with other common food spoilage microorganisms (*Bacillus stearothermophilus*, *Clostridium nigrificans* and *Clostridium pasteurianum*) indicate that food can be preserved with 100°C heat treatment for 10 minutes in the presence of 10 ppm oxytetracycline.

Uninoculated tubes containing peas, 1% glucose and 10 ppm oxytetracycline heated to 100°C for various lengths of time and stored at room temperature and 37°C showed that there is an initial loss of antibiotic activity on heating. No antibiotic activity could be demonstrated after the second week at 37°C and at the end of 5 weeks at room temperature.

The action of oxytetracycline in freshly ground chuck is bacteriostatic and decomposition of the meat closely follows that of the oxytetracycline.

Oxytetracycline as a food sterilization dip was evaluated for increasing the shelf-life of packaged leafy vegetables. Washed, chopped and shredded vegetables were slurried in water containing 30 ppm oxytetracycline for 10 to 15 minutes then drained and packaged into sterile jars. Vegetables after 30 ppm oxytetracycline dip showed a 30 to 100% increase in shelf-life.

In refrigerated custard filling, no oxytetracycline potency was lost in 120 hours. A 40% loss at room temperature and a 50% loss at 37°C was encountered.

No polymyxin activity was found in beer following treatment of the yeast with this antibiotic.

Oxytetracycline may be used at high levels to control salmonella contamination in dried egg products. Substantial residues result.

The residual level of procaine penicillin, DBED penicillin, streptomycin, oxytetracycline, chlortetracycline, carbomycin, bacitracin and polymyxin was tested in raw milk. All the antibiotics were stable for at least one week under refrigeration.

Bissett, H. M. and Tarr, H. L. A., 1952, Stability of antibiotics when used in experimentally retarding fish spoilage. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **93**, 23.

Chlortetracycline and oxytetracycline in minced coho salmon flesh at 2 and 5 ppm showed no potency loss after 4 days at 40°F. The antibiotics were stable in the presence of 200 ppm sodium nitrite and 40 ppm ascorbic acid. Heating the treated coho salmon and lingcod at 212°F inactivated over 50% of the antibiotics in 10 minutes and over 90% in 30 minutes.

Boyd, J., Brumwell, C. and Tarr, H. L. A., 1953, Aureomycin in experimental fish preservation. *Fisheries Research Board, Can., Progress Repts. Pacific Coast Stas.* **96**, 25.

It has been impossible to demonstrate residual antibiotic in the fish since some uncharacterized substances, naturally present in fish flesh, also give a zone of inhibition in the microbiological assay procedure.

Tolerance for residues of chlortetracycline. *Federal Register* **20**, 8776, Nov. 30 (1955).

Acceptance by the U. S. Food and Drug Administration of chlortetracycline for the treatment of uncooked poultry. A residue of the antibiotic not exceeding 7 ppm is permitted on any part of the poultry.

Barnes, E. M., 1956, Should antibiotics be used as food preservatives? American experience as a guide. *Food Manuf.* **31**, 508.

Against the undoubted efficacy of certain antibiotics in preserving some foods stands a number of objections: 1) unscrupulous processors might use antibiotics as a substitute for good hygiene; 2) antibiotics might eliminate the normal spoilage organisms and the food could be heavily contaminated with pathogens, but with no indication of this from off-flavors; 3) danger of the selection or development of resistant pathogens—particularly the food poisoning staphylococci and some salmonellas; 4) cumulative effect of inactive residues; and 5) extension of the use of medicinally important antibiotics may diminish their therapeutic value.

Boyd, J. W., Southcott, B. A. and Tarr, H. L. A., 1956–57, Antibiotic residues in fish iced with chlortetracycline ice and effect of normal cooking procedures on these residues. *Antibiotics Ann.*, 1002.

Chlortetracycline residues were determined in fresh, noneviscerated gray cod stored in ice containing 2 and 4 mcg/gm of the antibiotic. At intervals the fish were filleted, and the fillets (skinned and unskinned) were cooked to study the effects of baking and boiling on these residues. The antibiotic concentration was greatest in unskinned fillets, and tended to increase with storage time in ice. In a second experiment cooking residues were studied in gray cod that had been stored in ice containing 2.5 mcg/gm of the antibiotic. Both experiments showed that boiling removed all assayable antibiotic in skinned fillets, and usually most of that in unskinned fillets. Very small and variable concentrations (0.02 to 0.13 mcg/gm) of the antibiotic were present in baked fillets (skinned and unskinned) and in fried fillets. Samples of muscle and liver from finback whales which were treated postmortem by visceral injection of a chlortetracycline solution (100 gm/10 gal) showed very low

concentrations of the antibiotic, and no concentrations in quite a large proportion.

Durbin, C. G., 1956, Antibiotics in food preservation. *Am. J. Public Health* **46**, 1306.

Note of caution is made concerning use of antibiotics as a food preservative. Under the present Food, Drug and Cosmetic Act, antibiotics are pesticide chemicals when used as preservatives on or in raw agricultural commodities. Regulatory officials believe their safety has not been established for most proposed uses. Status of antibiotics in food in the United States is: 1) acceptable when no residues remain in the food; 2) acceptable when residues remain in the uncooked food, provided cooking always destroys all of the antibiotic; 3) under consideration when some antibiotic remains in the food as eaten. It must be ascertained that the addition of antibiotics to the food supply does not endanger the health of the consuming public.

Firman, M. C., Abbey, A., Darken, M. A., Kohler, A. R. and Upham, S. D., 1956, Effect of Aureomycin chlortetracycline on fish freshness. *Food Technol.* **10**, 381.

Freshness of fish—sea bass, weak fish, croaker, butterfish, scrod, porgy, salmon and halibut—was prolonged successfully by using an ice, a dip or a freezing brine containing chlortetracycline. Antibiotic activity which was determined in the raw fish ranged from 0.09 to 5.5 ppm, depending on treatment. Microbial counts and organoleptic observations confirmed the effectiveness of chlortetracycline in extending the storage life of freshly caught fish.

Henderson, G. N. and Hopwood, G. V., 1956, Antibiotics and food preservation. *Export Rev. Brit. Drug Chem. Inds.* **17**, 27.

Use of antibiotics in the preservation of edible food is not intended to replace normal refrigeration or freezing methods of preservation but merely to supplement them. Widespread use in meat preservation of antibiotics may increase the dangers of resistant organisms developing. Governments considering this process are necessarily cautious of the value and safety of the proposed products. It is suggested that some of the 2000 antibiotics which have already been discovered be studied for possible use in the preservation of food. There would be no possible dangers in using such an antibiotic if it did not exhibit similarities to antibiotics used therapeutically.

Kline, E. F., Abbey, A., Darken, M. A., Firman, M. C., Kohler, A. R., Miller, W. H., Wiley, W. W., Windlan, H. and Upham, S. D., 1956-57, Improved quality of fish fillets as a result of a food grade of chlortetracycline. *Antibiotics Ann.*, 997.

Whole red fish were treated with ices (5 ppm) and dipping solutions (25 ppm) containing chlortetracycline to prolong storage life. In the first experiment treated whole fish, graded at 14 days out of the sea by examination of eyes, gills, color, texture, odor and taste of the cooked fillet, were rated superior. Fillets of these fish were frozen into blocks and processed into fish sticks. Chlortetracycline assays of thawed samples before and after cooking showed higher residues in the skin than in the flesh; the highest level of 0.16 microgram chlortetracycline per gram of skin was completely destroyed by frying and reduced to 0.03 microgram by broiling.

A second experiment used higher concentrations (100 ppm) of chlortetracycline and longer times of dipping; all dip samples were stored in ice containing 5 ppm. Organoleptic tests and microbial counts of whole fish and of thawed fillets showed treated fish to be superior. Frying tests reduced the chlortetracycline residue to zero and broiling significantly reduced residues. In fish stored 10 days on the boat in ice containing chlortetracycline, 30% were rated as excellent and 65% as very good, compared to 10% and 45% in control fish, respectively.

Shirk, R. J., Whitehill, A. R. and Hines, L. R., 1956-57, A degradation product in cooked Aureomycin chlortetracycline treated poultry. *Antibiotics Ann.*, 843.

Isochlortetracycline is formed when chlortetracycline-treated poultry is heated. Isochlortetracycline was showed to be a normal degradation product of chlortetracycline in the gastrointestinal tract of the rat. The compound is microbiologically inactive and has an oral LD₅₀ greater than 10 grams per kilogram in mice.

Steiner, G. and Tarr, H. L. A., 1956, Penetration of chlortetracycline into fish muscle and its destruction by heat. *Can. J. Technol.* **34**, 215.

Concentrations of chlortetracycline were determined by microbiological assay in flesh of gray cod iced with ice containing 1 mcg/gm of the antibiotic, or held in refrigerated sea water containing 2.8 mcg/gm. In iced fish, relatively small amounts penetrated the fish, largely taking place in the visceral cavity walls. Fish stored in sea water containing 2.8 ppm chlortetracycline absorbed more antibiotic than those stored in ice containing 1 ppm chlortetracycline; the skin and visceral cavity walls also had the highest concentrations. Destruction of chlortetracycline in fish flesh was determined in samples heated to 60°, 82° and 99°C.

Tolerance for residues of oxytetracycline. *Federal Register* **21**, 8104, Oct. 23 (1956).

Acceptance by the U. S. Food and Drug Administration of oxytetracycline for the treatment of uncooked poultry. A residue of the antibiotic not exceeding 7 ppm is permitted on any part of the poultry.

Bacharach, A. L., 1957, Antibiotics and hazards. *Food, Drug, Cosmetic Law J.* **12**, 505.

The present legal position is summarized of the use in Great Britain of antibiotics for any purpose. The hazards are enumerated as likely to be due to the presence of antibiotics in food for humans—toxicity, carcinogenicity, allergy, drug resistance.

Goldberg, H. S., Read, B. E. and Goodman, R. N., 1957-58, Studies on the emergence of streptomycin-resistant bacteria as a result of low-level, long-term feeding of streptomycin. *Antibiotics Ann.*, 144.

Emergence of resistance to streptomycin depended primarily on the dosage level and type of bacteria. Coliforms did not emerge resistant in mice fed 0.0 mcg/mouse/day. Coliforms emerged resistant in mice fed 20 and 40 mcg/mouse/day, the peak occurring three to four months after the start of feeding and dropping off. The level of resistance with 20-mcg feeding did not

go above 10 mcg/ml, but feeding at 40 mcg produced many coliforms resistant to 1000 mcg/ml. Oral micrococci did not emerge resistant in guinea pigs fed 35 mcg/pig/day. Emergence of resistance appears to vary for each antibiotic, each dosage, each type of bacterium and each host. Long-term feeding studies on various experimental animals are necessary to determine the public health significance of antibiotic residues.

Logue, J. T., Goldberg, H. S. and Goodman, R. N., 1957-58, The public health significance of antibiotic residues in food. *Antibiotic Ann.*, 333.

A planned program of investigation is needed to determine which antibiotic is most suitable to food treatment and preservation and at what levels. This information can be applied experimentally in animals and humans to determine potential public health hazards. Preliminary data are presented of determining hypersensitivity of field workers spraying streptomycin on fruits and vegetables. Of 30 workers who had used streptomycin sprays frequently over three to four years, 2 gave a strongly positive skin test to streptomycin. In 50 normal adult controls none showed a positive reaction to streptomycin.

Morrell, C. A. and Thatcher, F. S., 1957, Antibiotics in foods. *Food, Drug, Cosmetic Law J.* 12, 477.

Use of antibiotics in foods requires limitations and other measures to prevent the degradation of their value in medicine and to assure that the resulting food products are safe. The article discusses the practical and public health issues as related to the Food and Drugs Act of Canada.

Partmann, W., 1957, Antibiotics in food preservation (in German). *Z. Lebensm.-Untersuch. u.-Forsch.* 106, 210.

An extensive and detailed review of work in the field is presented in this article. Use of chlortetracycline in processing and storage of fish, poultry and mammals is successful. Toxicological tests and medical therapy tend to show that the antibiotic is not harmful. In feeding tests with a constant intake of chlortetracycline fat synthesis in animals has increased with a decrease in that of protein. Chlortetracycline apparently can act in the enzyme systems of different organisms having a common metabolism. Antibiotic levels in foods decrease during storage. Chlortetracycline in meat from warm-blooded animals is destroyed during cooking; the level in fish is reduced about two-thirds. Such foods are considered safe after cooking and the low antibiotic level remaining probably will not cause emergence of resistant strains of human pathogenic organisms. The maximum concentration of chlortetracycline in such foods must be determined and whether destruction products of this compound are harmful to humans.

Pugsley, L. I., 1957, Chemical preservation of foods. *Can. Chem. Processing* 41, 50.

The bacteriostatic, fungistatic or fungicidal properties of Class I, II and III food preservatives as defined by Canada are discussed. Considerable attention has been given to the use of antibiotics as antimicrobial agents in foods. In 1952 Tarr and his associates at Vancouver reported the effectiveness of chlortetracycline for increasing the storage life of fish. The possibility of small quantities of antibiotic residues to cause sensitization and of the emergence of resistant strains of bacteria has been considered. Chemical preservatives

should never be used as a substitute for cleanliness or to disguise inferior raw material. A rigid policy is maintained to limit the list of chemicals used in food, to demand evidence of their harmlessness, and to determine that in their use a useful purpose will be served in the interests of the consumer.

Tomiyama, T., Yone, Y. and Mikajiri, K., 1957, Penetration of chlortetracycline into fish flesh or round fish and its heat inactivation (in Japanese). *Bull. Japan. Soc. Sci. Fisheries* **22**, 778.

A modified pad-plate method was used to determine the distribution pattern of chlortetracycline in treated fish and the rate of its inactivation by heat. Several species of round fish were stored 27 hours in chilled sea water containing 100 ppm citric acid and 10 ppm CTC. Only pilchard and small mackerel showed penetration of CTC into the flesh. In "isaki" under the same conditions no CTC was found in the flesh or viscera but was present in the skin and gills. CTC readily penetrated bonito fillet and its rate of heat inactivation increased with rise in temperature and length of heating.

Tomiyama, T., Yone, Y. and Mikajiri, K., 1957, Uptake of Aureomycin chlortetracycline by fish and its heat inactivation. *Food Technol.* **11**, 290.

Uptake, distribution and heat destruction of chlortetracycline in four species of round fish and in fillets were determined by a modified pad-plate assay. After 27 hours the antibiotic in chilled sea water (10 ppm) did not penetrate into muscle tissue of round fish except in small fish with incompletely developed scales (pilchard and common mackerel). Fresh round fish "isaki" stored under the same conditions showed a measurable uptake of CTC in the skin and gills but none into the flesh or viscera. Bonito fillets stored in chilled sea water containing 10 ppm CTC for five hours picked up a small amount near the surface but none penetrated deeper than 10 mm. Rate of heat destruction of CTC was increased with rise in temperature and length of heating.

Welch, H., 1957, Antibiotics in food preservation. Public health and regulatory aspects. *Science* **126**, 1159.

The antibiotics used in preservation of perishable foods are only one group of the expanding list of chemicals that may find their way into our food supply. The actual or potential introduction of antibiotics has magnified the problems of the U. S. Food and Drug Administration when considering side reactions and possible significance to public health. About 10% of our population has a tendency to become sensitive to some food, drug, cosmetic or other substance. Use of antibiotics in animal feeds for growth stimulation and for prophylactic or therapeutic purposes does not constitute a public health hazard. Antibiotics used as crop sprays are dissipated and there is no public health problem. Antibiotics may be introduced indirectly into foods as in milk from cows being treated for mastitis. Presence of the antibiotic in the milk is deemed an adulteration. A tolerance level of 7 ppm of chlortetracycline or oxytetracycline in raw poultry is allowed because no significant antibiotic residues could be found in the poultry after cooking by broiling, frying, boiling or baking. In considering a tolerance level of these antibiotics in fish, it has been found that ordinary methods of cooking treated fish do not eliminate the residual antibiotic. It will be necessary to demonstrate that the residues are not dangerous to public health.

Welch, H., 1957, Control of antibiotics in food. *Food, Drug, Cosmetic Law J.* **12**, 462.

Antibiotics are used in animal nutrition for promotion of growth; they are used as crop sprays to prevent blight and bacterial disease; therapeutic and prophylactic use of antibiotics is made in animals; and extensive research has explored the potentialities of antibiotics as food preservatives. Tolerance for chlortetracycline in the preservation of raw poultry was granted. The indirect introduction of antibiotics into the food supply is deemed an adulteration. The public health problem of antibiotics, particularly penicillin, in the milk supply is great and the U. S. Food and Drug Administration has taken three steps to alleviate it.

Bottomley, R. A., 1958, Food additives—preservatives, antioxidants and antibiotics. *Food Technol. in Australia* **10**, 63.

Food additives must be to the consumer's benefit as defined by the FAO/WHO Committee and can never substitute for poor technology or poor sanitation. There appears to be sufficient evidence for extension in Australia of the list of food preservatives and for introduction of some antibiotics for prescribed foods. Difficulty of control and the possible side effects of medical importance require further evidence for the use of antibiotics. It is likely that antibiotics of no medical significance can be used. Nisin, for example, occurs naturally in certain cheeses, is nontoxic and nisin-resistant bacteria are not cross-resistant to the medical antibiotics. Besides its use in processed cheese, nisin also appears to find application in canning as a supplement to heat treatment.

Escanilla, O. I., Carlin, A. F. and Ayres, J. C., 1958, Studies on residual chlortetracycline in meat. Presented 18th Ann. Meet., Inst. Food Technologists; Abstr., 1958, *Food Technol.* **12**, 46.

Microbiological assays determined the residual chlortetracycline in raw and cooked round steak, ground beef and wieners. Round steak was infused with the antibiotic (5 mcg/gm) and broiled to an internal temperature of 60°C. An appreciable amount of antibiotic remained in the cooked meat and the extent of inactivation was not the same throughout the steak. When one-inch cubes of steak were stewed approximately two hours, the antibiotic was completely inactivated. Chlortetracycline was added to ground meat at levels of 15, 5 and 1.7 mcg/gm. Assays were made on raw samples stored one day (8°C) and on meat patties broiled to rare, medium and well-done stages. Wieners containing the antibiotic at levels of 30, 10 and 3.3 mcg/gm were smoked before storage at 1°C for 26 days. Assays were made at intervals on raw samples and on wieners which remained in boiling water for 10 minutes. Loss in antibiotic content during storage was gradual.

Thatcher, F. S., 1958, Antibiotics in foods: a review of some public health aspects. *Can. J. Public Health* **49**, 58.

A number of conclusions are presented pertinent to the presence of antibiotics in foods for use in Canada. Necessary properties are listed in detail for those antibiotics likely to satisfy reasonable requirements for use as preservatives in foods. The problems of antibiotic residues and antibiotic-resistant staphylococci in milk seem inseparably associated with the control of mastitis and action is needed.

G. ECONOMIC AND OTHER BENEFITS

Joint FAO/WHO Expert Committee on Food Additives, 1957, General principles governing the use of food additives. World Health Organization, Tech. Rept. Ser., No. 129.

The points are enumerated which must be considered by public health authorities in establishing regulations to govern the use of food additives. It is pointed out that socio-economic status is an important factor; that lack of modern facilities may increase the need; that high temperature and humidity favor deterioration and justify greater use of additives than in more temperate climates. Possible risks must be weighed against benefits to consumer.

Wrenshall, C. L., 1956-57, Advances in food technology made possible through the use of antibiotics. *Antibiotics Ann.*, 809.

General review is presented of the role of antibiotics in preservation.

CHAPTER VI

ANTIBIOTICS IN THE ISOLATION AND CULTIVATION OF MICROORGANISMS

BY HERBERT S. GOLDBERG

A. INTRODUCTION

Selective agents have been incorporated into media by microbiologists since the time of Ehrlich. Dyes, organic acids and other inorganic and organic reagents have been advocated for selective inhibition of one group or another of undesirable microorganisms.

Need for such selective control arises from the fact that most common bacterial and mold contaminants are less fastidious, more resistant, and more adaptable to environment than most plant and animal pathogens. Moreover, the clinical or research worker rarely has a specimen that contains the desired organism in pure culture. He must separate, distinguish and identify a desired culture occurring among a multitude of closely-related microorganisms. Furthermore, his problem is often complicated by the use of lengthy incubations that subject his culture media to airborne contaminants of a bacterial and fungal nature.

In his original paper on the discovery of penicillin, Fleming¹ discussed this problem in the cultivation of *H. influenzae*, and he suggested that penicillin be incorporated in a selective medium for isolation of this organism. Since that time antibiotics have been used successfully for selective microbial control in many other instances.

Nutritional and metabolic studies on protozoa and related organisms have in general lagged considerably behind similar work on bacteria. This delay has been attributed to difficulties in (1) supporting growth of protozoa in pure culture without contributory activities of bacteria, and (2) preparing chemically defined media whereby growth requirements can be analyzed both qualitatively and quantitatively.² Antibiotics incorporated into protozoan cultures have contributed immeasurably to the solution of the first, i.e., the bacterial aspect of this problem. Such uses of antibiotics have made possible contributions to the study of *Trichomonas*, *Endamoeba*, haemoflagellates and even cestodes and nematodes. Some of these applications of antibiotics to protozoology are reviewed in detail below.

Another major use of antibiotics in selective isolation and cultivation of microbes has been in the application of antibiotics to tissue culture and chick embryo culture techniques in the study of rickettsia and viruses. Moreover, antibiotics are used to handle specimens on primary isolation before the cultivation of these organisms. This use in isolation is so widespread today that it is difficult to imagine the isolation of, for example, polio virus from feces without using penicillin and streptomycin! Though possible, it would involve cumbersome filtration methods and specialized centrifugation procedures not readily available at many installations.

The use of antibiotics in viral and rickettsial isolation and cultivation is discussed more fully later in this chapter. There, some unusual combinations of antibiotics which have been cleverly devised by clinical laboratory and research workers for this purpose are discussed, with references to the literature.

One of the most striking and useful applications of antibiotics in culture media has been in isolation of fungi. Recently awakened interest in fungal diseases of plants, animals and humans has been responsible for the progress in development of newer and better growth media. They are often based upon the antibiotic, cycloheximide (actidione), which has been found to inhibit many yeasts, molds and saprophytic fungi.³ In addition, its activity against bacteria and certain pathogenic fungi is very low. This antibiotic is quite complementary to the activity of penicillin and streptomycin, and thus these three in combination can eliminate most of the organisms that are undesirable in the isolation of pathogenic fungi.

Another use of antibiotics in selecting fungi occurs in screening for antibiotic producers from soil samples. The myriad of organisms in the soil easily mask the *Streptomyces*, which are the main source of antibiotics. Later in this chapter a review is given of the application to soil screening procedures of selective media containing antibiotic combinations.

B. EVALUATING ANTIBIOTICS

In attempting to establish a combination of antibiotics that will be effective in selective media, one has to consider most seriously several potential hazards. The antimicrobial spectrum is only one of the criteria. The physical and chemical properties of the antibiotic are also very important. For example, chlortetracycline, a wide spectrum antibiotic, is very unstable.⁴ Table 6-1 summarizes data on its loss of potency in various bacteriological media at 37°C at pH 7.0.

These figures warrant the conclusion that this antibiotic can have

TABLE 6-1
LOSS OF POTENCY OF CHLORTETRACYCLINE IN
VARIOUS BACTERIOLOGICAL MEDIA

Material tested	Concentration, per cent	Per cent loss in potency	
		5 hours	24 hours
Penassay broth	—	43	96
Beef extract	0.15	35	90
Peptone	.5	24	76
Phos. buffer pH 7.0	.5	56	89
Fl. thioglycollate	whole medium	32	60
Casitone	.15	25	80
Yeast extract	.5	40	80
Dextrose	.5	8	46
Sodium chloride	.25	25	82
L-cystine	.1	20	51
Na thioglycollate	.05	20	50
Trypticase	1.5	20	55
Thiol medium (Difco)	3.0	25	98
N-A case peptone	1.5	37	90
Water, distilled	—	15	46

Price *et al*, 1948.
Ann. N. Y. Acad. Sci. **51**, 213.

relatively little use in selective culture media. Other important properties besides stability are solubility, thermal stability and agar diffusability. Any of these properties may eliminate an antibiotic which has the desired antimicrobial spectrum from use in a selective medium.

Another important property in evaluating an antibiotic is its toxicity to, or other injurious effects upon, the tissues used in tissue culture and chick embryo cultivation of viruses and rickettsia. For example, specific tissue toxic effects are exhibited by streptomycin and certain other antibiotics. Many studies have been carried out to evaluate the toxic effect of antibiotics on culture tissues, chick embryos and viruses to be cultured therein. These reports are reviewed by Brandly and Winslow.⁵

The successful use of antibiotics in selective culture media is reviewed with detailed references in the following sections on bacteria, fungi, viruses and protozoa. Because much of the data, particularly that on penicillin and streptomycin in virus cultivation, is found repeatedly in the literature, no attempt has been made to present all the literature in the field. Rather this chapter aims to provide representative examples of useful, diversified, nontherapeutic functions of antibiotics in microbiology, thereby fulfilling the objective of this book.

C. BACTERIA—CULTURE MEDIA USING ANTIBIOTICS

Prior to his discovery of penicillin, Sir Alexander Fleming had great interest in the organism *B. influenzae* (*Haemophilus influenzae*). Therefore it is not surprising that in his original report on the isolation, characteristics, and properties of penicillin, he should suggest this antibiotic as an aid to cultivating *H. influenzae*.¹ He proposed the use of penicillin on plates streaked with sputum, throat swabs, etc. for the isolation of this organism. Shortly after this first penicillin report, he published a paper devoted to the occurrence of influenza bacilli in the mouths of normal people. Throughout this work he was able to isolate para-influenza bacilli from the human gums and tonsils, using penicillin selective media.⁶ Pursuing this work even further in 1932, Fleming published a discussion of the spectrum properties and methods of using penicillin and potassium tellurite in selective media.⁷ He noted that these agents act on different bacterial species, and he gave further evidence for use of penicillin in isolation of *H. influenzae*.

These papers advocating the use of penicillin for isolating hemophilic organisms were widely read and adopted. In 1937 Maclean modified the standard cough plate method for the diagnosis of pertussis. He utilized penicillin and obtained many positive cultures.⁸ Penicillin continued to hold favor in the isolation of hemophilic organisms as evidenced by succeeding reports by Buxbaum and Fiegoli⁹ and Cruickshank.¹⁰ The Buxbaum and Fiegoli report concerned itself with laryngeal cultures of *H. influenzae* from obstructive laryngitis. In comparing blood agar plus penicillin, blood agar plus sodium oleate, and plain blood agar, it was found that the antibiotic-containing media gave most positive cultures.

On the other hand, Cruickshank found that in cultivating pertussis bacilli the Bordet-Gengou plate was as good as a penicillin-containing medium. It has been suggested that these results were complicated by penicillin-resistant contaminants, and therefore increased positive cultures of pertussis did not occur.¹¹ Since the report of Cruickshank many subsequent papers have indicated the usefulness of penicillin added to Bordet-Gengou plates. See Bradford,¹² Lacey¹³ and Lacey.¹⁴

Other antibiotics have been substituted for penicillin in cultivation of hemophilic organisms. Schoenback and Seidman devised a selective medium for *H. influenzae*, using tyrothricin.¹⁵ They studied chocolate agar and Fildes medium with and without the antibiotic. The tyrothricin media gave superior results.

Following successful cultivation of hemophilic bacteria with antibiotics as originally suggested by Fleming, there appeared a number of studies of

various antibiotics in selective cultivation of various bacteria. They included "acne" bacilli, brucella spp., tubercle bacilli and clostridial spp.

Craddock included penicillin in glucose broth for cultivation of acne lesions. He observed a one hundred per cent isolation of "acne bacilli" (gram positive diphtheroid-like rods) with penicillin and somewhat less without.¹⁶

A series of reports advocating the use of antibiotics for selective isolation of *Brucella* species have recently appeared.

Kuzdes and Morse suggested a medium for isolating *Brucella* from soil, manure, water, milk and aborted fetuses. The medium contained Polymyxin B, cycloheximide, bacitracin, circulin and crystal violet. On control studies the normal contaminants of these specimens overgrew *Brucella*.¹⁷

In 1955 Morris described a medium containing 100 $\mu\text{g}/\text{ml}$ cycloheximide, 6,000 units/ml Polymyxin B and 5,000 units/ml penicillin for isolating *Brucella* from herd samples of milk. This report was followed by Morris with a selective medium for *Brucella* cultivation from feces. The medium contained 5-nitrofurfuryl-methyl ether, α -nitro furan, bacitracin, polymyxin and cycloheximide. This medium grew *Brucella* from above specimens in 65 hours.¹⁸

Isolation of the tubercle bacillus has always been a problem because of the heavily contaminated specimens in which it is found. Tarshis *et al* have reported a blood medium for the cultivation of *M. tuberculosis*. They compared blood agar-penicillin and Lowenstein-Jensen media under routine conditions. They concluded that the blood-antibiotic gave more positives with shorter incubation time.¹⁹ (See Table 6-2).

Another group of organisms most often found in heavily contaminated specimens are members of the genus *Clostridium*. Wetzler, Marshall and Cardella used a combination of sorbic acid and Polymyxin B in a liquid medium. They found this medium would inhibit aerobic organisms as usually encountered in mixed cultures from war wounds. The *Clostridia* grew out very well.²⁰

The fermentation industries have recently solved some of their problems by use of antibiotic selective media. Beech and Carr report use of cycloheximide in isolating bacteria from apple juices.²¹ Cycloheximide has been found functional in enhancing counting of bacterial contaminants in brewery yeasts and beers.^{22,23} This is particularly true since the yeasts (*Saccharomyces*) are highly sensitive to this antibiotic.

A survey of the use of antibiotics in selective growth of bacteria would appear to reveal the following: penicillin is by far the most important antibiotic in that it has a specific gram positive spectrum, it is stable in

TABLE 6-2

COMPARISON OF BLOOD AGAR-PENICILLIN AND LOWENSTEIN-JENSEN MEDIA UNDER ROUTINE DIAGNOSTIC CONDITIONS

A. General analysis of specimens

Total number of specimens.....	1,012
Total number of positives.....	378
Total number of negatives.....	568
Total number contaminated.....	66

B. Comparison of growth on different media

	Number	Per cent
Positive blood, positive Lowenstein-Jensen.....	316	83.6
Positive blood, negative Lowenstein-Jensen.....	38	10.1
Negative blood, positive Lowenstein-Jensen.....	24	6.3
Total positives, blood.....	354	93.6
Total positives, Lowenstein-Jensen.....	340	89.9

C. Comparison of rate of growth

Average number of days first appeared, blood.....	18.9
Average number of days first appeared, Lowenstein-Jensen	21.5

Time of first appearance (in days)	Blood		Lowenstein-Jensen	
	Number	Per cent	Number	Per cent
1-7	8	2.3	1	0.3
8-14	91	25.7	84	24.7
15-21	155	43.8	142	41.8
22-28	56	15.8	63	18.5
29-35	19	5.4	28	8.2
36-42	21	5.9	15	4.4
42	4	1.1	7	2.1

D. Comparison of contamination

	Number	Per cent
Total number of tubes of each medium.....	2,024	
Number of tubes contaminated, blood.....	102	5.0
Number of tubes contaminated, Lowenstein-Jensen.....	140	6.9

Tarshis *et al*, 1953.

J. Bact. **66**, 448-452.

the presence of bacteriological media as contrasted to chlortetracycline, and it is readily available.

Of the gram-negative inhibitors, Polymyxin B appears to be the antibiotic of choice for this purpose. However, any narrow spectrum antibiotic could be considered useful as a potential selective agent.

The circumstances which appear to favor the use of these materials are heavy contamination of the specimens, and cultures requiring incubation for a long time. Frequently the use of antibiotics with dyes or chemicals provides a better inhibitory result than the antibiotics alone.

D. FUNGI—CULTURE MEDIA USING ANTIBIOTICS

Recent clinical developments in study of infectious disease have made it increasingly desirable to improve culture methods for fungi pathogenic for man. In addition, plant pathologists have encountered extreme difficulty in studying some plant disease fungi because of inadequate cultural methods.

For the most part clinical laboratories have relied on Sabouraud's dextrose agar, and plant pathologists have depended on potato dextrose agar for cultivation of fungi.

The problems existing in the isolation of specific fungi are manifold. Two are especially significant. First, the fungi grow slowly so that plates must be incubated for 1-8 weeks.²⁴ Second, the culture must usually be incubated at room temperature to elaborate the mycelial phase which is of diagnostic significance. As a result, conditions are favorable for contamination by both bacterial and fungal saprophytes.

The advent of antibiotics with antibacterial spectra resulted in the establishment by several investigators of selective media for fungi which contained antibiotics to inhibit the bacteria. In 1945 Thompson²⁵ noted a specialized blood agar medium containing penicillin and streptomycin for isolation of human pathogenic fungi. A more detailed report on the use of the same antibiotics was produced two years later by Boeing and Laffer.²⁶ However, effective isolation of fungal pathogens did not become a routine procedure until Littman and his group outlined an entirely new medium in a series of three detailed reports. In the first report²⁷ Littman described an oxgall agar containing crystal violet (1 : 100,000), an old standby of Ehrlich's days, and streptomycin in the amount of 30 μ g/ml which permitted isolated clonial growth of saprophytic and pathogenic fungi. This was followed shortly by another study comparing this new medium with Sabouraud's dextrose agar. In a series of tests on 47 specimens the oxgall-dye-antibiotic agar was shown to detect four times as many pathogenic fungi as did Sabouraud's medium.²⁸ In a final evaluation of this antibiotic-containing medium under routine conditions at a large diagnostic laboratory and clinic, its value was firmly established. In tests of 384 specimens cultured over 12 months, cutaneous mycoses were diagnosed five times more frequently than with Sabouraud's dextrose, while systemic mycoses were detected twice as frequently.²⁹

In the late 1940's an antibiotic was discovered which was to have great influence on cultivation of fungi. In a series of reports^{30,31} cycloheximide was described as having a most unusual antimicrobial spectrum. It has low activity against bacteria and actinomycetes, and it inhibits

many yeasts and most saprophytic fungi. Thus it was clearly the perfect antibiotic to combine with penicillin and streptomycin in cultivation of pathogenic fungi. Therefore, cycloheximide falls into that group of antifungal agents, described by Waksman *et al.*,³² that are active against fungi but not against bacteria or actinomycetes. Evaluating this antibiotic for the control of mold contaminants on cultivation of bacterial plates, Phillips and Hanel³³ found that 100 $\mu\text{g/ml}$ in an agar medium inhibited 7 genera (12 species) of fungi that are common contaminants. In addition this concentration had no effect on 27 species of pathogenic bacteria.

In attempting to devise a selective medium for the isolation of *Coccidioides immitis*, George, Ajello and Gordon³⁴ took advantage of the complementary activity of streptomycin, penicillin and cycloheximide. Using concentrations of streptomycin of 20 $\mu\text{g/ml}$, penicillin of 40 $\mu\text{g/ml}$ and cycloheximide of 100 $\mu\text{g/ml}$ in Sabouraud's dextrose agar, they were able to isolate *C. immitis* from contaminated sources. Similar results using cycloheximide were obtained by Fuentes *et al.*,³⁵ who indicated reduction of the number of saprophytic fungi on their plates.

A recent survey of the effect of cycloheximide on fungi pathogenic to man was carried out by George *et al.*³⁶ They found that at the concentration commonly employed (100 $\mu\text{g/ml}$) only *Cryptococcus neoformans*, *Allescheria boydii* and *Aspergillus fumigatus* were inhibited by cycloheximide.

In 1957 Rosenthal and Furnari devised a medium for the routine culture of fungi in which they substituted a broad spectrum antibiotic, chloramphenicol, for streptomycin and penicillin, which they combined with cycloheximide. They investigated dermatophytic infection in 600 patients. They obtained twice as many isolations with chloramphenicol-cycloheximide agar as they did with Sabouraud's dextrose agar. Contamination was reduced by 50%.³⁷ Many other workers have utilized antibiotics, particularly cycloheximide, in isolation of dermatophytes by use of an antibiotic medium rather than by a medium without antibiotics.³⁸ See Table 6-3. Sharvill and Talbot and Ajello and Getz had comparable results when studying human superficial mycoses.^{39,40}

One of the problems in isolation of fungi from clinical materials concerns those pathogens which are most common in the yeast phase. These cultures grow well on blood agar at 37°C, but usually must be isolated from newly contaminated sources, such as sputum.

In 1956 Marcus, Nielson and Rambo reported on an antibiotic-blood-agar used effectively for isolation of *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Cryptococcus neoformans* from sputum. The medium

TABLE 6-3

ISOLATION OF *TRICHOPHYTON VERRUCOSUM* FROM CATTLE HAIRS:
COMPARISON OF ISOLATION OF *T. VERRUCOSUM* ON SABOURAUD-
PENICILLIN-STREPTOMYCIN AGAR, WITH AND WITHOUT
CYCLOHEXIMIDE AND WITH CYCLOHEXIMIDE
AND THIAMINE

Specimen No.	6 Tubes without cycloheximide			6 Tubes with cycloheximide			6 Tubes with cycloheximide and thiamine		
	Positive for <i>T. verrucosum</i>	Negative*	Saprophyte contamination†	Positive for <i>T. verrucosum</i>	Negative*	Saprophyte contamination†	Positive for <i>T. verrucosum</i>	Negative*	Saprophyte contamination†
1	0	0	6	4	2	0	5	1	0
2	6	0	0	6	0	0	6	0	0
3	1	2	3	3	3	0	2	4	0
4	0	0	6	3	2	1	4	0	2
5	2	0	4	6	0	0	6	0	0
6	0	0	6	1	3	2	2	4	0
7	2	0	4	6	0	0	5	0	1
8	2	0	4	6	0	0	6	0	0
9	0	0	6	4	0	2	5	1	0
Totals	13	2	39	39	10	5	41	10	3

* Number of tubes in which no dermatophyte developed and saprophytic contamination was either absent or minimal by the 10th day.

† Number of tubes in which more than one-half of slant was covered with saprophytic growth by the 10th day.

Georg, L. K., 1953.

Arch. Derm. & Syph. **67**, 355-361.

contained penicillin, streptomycin and Polymyxin B. They had good qualitative and quantitative results.⁴¹

E. SOIL-PLANT FUNGI—CULTURE MEDIA USING ANTIBIOTICS

Whiffen, one of the co-discoverers of cycloheximide, has made a study of the *in vitro* activity of the antibiotic on 33 species of fungi pathogenic to plants.³¹ She first used a medium containing 100-300 µg/ml of streptomycin to isolate the fungi. She then tested sensitivity of these cultures to cycloheximide incorporated in agar at levels of 0.1 µg/ml-100.0 µg/ml. Inhibitory activity was observed against 1 order of Phycomycetes, 4 orders of Ascomycetes, 1 order of Basidiomycetes and 12 genera of *fungi imperfecti*.

On the other hand, Jeffers⁴² in a study on phytopathogenic bacteria showed that 1,000 $\mu\text{g}/\text{ml}$ of cycloheximide would not inhibit these bacteria.

Two recent papers have been reported concerned with the isolation of *Graphium ulmi*, the etiologic agent of Dutch Elm disease. In 1955 Holmes⁴³ showed the value of cycloheximide in controlling mold contamination during the isolation of *G. ulmi*. In 1956 Schneider⁴⁴ reported on a selective medium for the isolation of *G. ulmi*. The medium was potato dextrose agar, plus 10 $\mu\text{g}/\text{ml}$ streptomycin and 200–300 $\mu\text{g}/\text{ml}$ cycloheximide. It was determined that this medium would inhibit *Penicillium* sp., *Aspergillus* sp., *Cephalosporium* sp., *Fusarium* sp., and other common contaminants. Growth of *Graphium ulmi* was not inhibited at all.

It has always been the desire of the agronomist, soil microbiologist and plant pathologist to learn more about the microbial population of the soil. Of recent date this desire has been intensified by the frequency with which antibiotic-producing organisms have been isolated from soil samples. By judicious use of various antibiotics incorporated in special media, it is now possible to isolate specific groups of organisms selectively from this environment.

Martin in 1950⁴⁵ made a comparative study of the use of acids, rose bengal dye and streptomycin as bacterial growth inhibitors for determining numbers and kinds of soil fungi by the plate dilution technique. After comparing these agents singly and in various combinations, he found that a medium containing 1 : 30,000 rose bengal and 30 $\mu\text{g}/\text{ml}$ streptomycin in potato dextrose agar gives the best results for plating of soil fungi. This medium increased the number of fungi by 100% and the number of types of fungi by 14%.

More recently, Hine made a similar study and substituted in Martin's medium above, novobiocin 100 $\mu\text{g}/\text{ml}$ for streptomycin. This change resulted in better qualitative results, with 75 different species of fungi producing colonies on Hine's medium.⁴⁶

Reports of antibiotic screening procedures from soil samples include a number of attempts at selective isolation of streptomyces as a process in antibiotic production. Dulaney, Larsen and Stapley eliminated undesirable organisms and isolated streptomyces in a medium containing cycloheximide, polymyxin, subtilin and penicillin. To eliminate actinomycetes and bacteria in a search for fungi, they used penicillin, streptomycin, polymyxin, bacitracin, and chlortetracycline.⁴⁷ Johnson found tetracycline antibiotics best for isolating soil fungi.⁴⁸ He tested 8 antibiotics for their ability to inhibit soil organisms without reducing fungi, and he found that the tetracycline was best. Corke and Chase,

in an attempt to select actinomycetes and inhibit fungi, made a study of sodium propionate and cycloheximide. They found the antibiotic superior to the organic acid derivative. Using 100 $\mu\text{g}/\text{ml}$ of cycloheximide, they inhibited fungi but could culture 85 different actinomycetes.⁴⁹

In a highly specific medium Silvestri⁵⁰ was able to enhance the growth of tetracycline-producing streptomyces. Using 30 $\mu\text{g}/\text{ml}$ tetracycline and 1 : 10,000 paraben, he inhibited mold and bacterial growth, and he gave a selective advantage to the tetracycline-producing streptomyces.

In summing up the use of antibiotics for selective cultivation of fungi and actinomycetes, the conclusion appears warranted that the single antibiotic most effective for this purpose is cycloheximide. In its ability to inhibit yeasts and saprophytic fungi while permitting plant and animal pathogenic fungi to grow, plus its lack of inhibition for bacteria at even 1,000 $\mu\text{g}/\text{ml}$, cycloheximide is unmatched among other antibiotics. By combining this very useful agent with broad spectrum antibacterial antibiotics such as penicillin-streptomycin, or chloramphenicol, a highly selective medium for fungal and actinomycete culture is made available.

F. PROTOZOA AND RELATED ORGANISMS—CULTURE MEDIA USING ANTIBIOTICS

The advantages of having an organism available for pure culture study are well established. One cannot clearly indicate the pathogenic properties of an organism nor study the biological characteristic of a given species in a mixed culture. Therefore, the maintenance of protozoa and related organisms growing symbiotically with bacteria is undesirable. Many investigators have tried to free protozoa from other forms of microbial life. The literature refers to a great number of techniques including centrifuging, washing, micropipetting and addition of disinfectants and germicides.⁵¹ On those occasions when a bacterial-free culture was obtained, the medium used would not be growth-supporting.

The protozoan studied earliest along these lines was *Trichomonas vaginalis*. Trussel, in an excellent study, first isolated and maintained a bacterial-free culture of *T. vaginalis* on a medium of liver infusion agar, human serum and rice powder.⁵² Maintenance of this culture was dependent on strict aseptic techniques and was subject, therefore, to contamination. However, in 1944 Adler and Pulvertaft inoculated 3 contaminated cultures of *T. vaginalis* into a special medium containing 90 $\mu\text{g}/\text{ml}$ of penicillin and obtained 3 bacteria-free strains of *T. vaginalis*.⁵³

In 1945 a study was made in a similar direction using penicillin, sulfathiazole and tryothricin in an attempt to get bacteria-free isolations

of *T. vaginalis*. Penicillin at 1,000 $\mu\text{g}/\text{ml}$ gave best results. Sulfathiazole plus penicillin gave no improvement over penicillin alone, and tyrothricin plus penicillin was lethal to the trichomonads.⁵⁴

When streptomycin was available, Quisno and Foter purified *T. vaginalis* cultures from bacteria by the addition of 25 $\mu\text{g}/\text{ml}$ streptomycin after 10 hours at 37°C.⁵⁵

These techniques have also been applied to *T. foetus* by several investigators in studies to determine what antibiotics were most useful in selective media for protozoa.^{56,57} Morgan combined penicillin and streptomycin at levels of 50,000 $\mu\text{g}/\text{ml}$ and 1,000 $\mu\text{g}/\text{ml}$, respectively, with good success in growth of *T. foetus*.⁵⁷ The use of 100 $\mu\text{g}/\text{ml}$ each of penicillin and streptomycin controlled all bacterial contaminants in a study by Williams and Plastringe.⁵⁸ They also found clavacin, gramicidin, and actinomycin to be toxic to trichomonads.

Another important group of protozoa that have been investigated frequently for growth characteristics are the amoebae, particularly *Endamoeba histolytica*. In 1946 a study was reported on the effect of streptomycin on *E. histolytica* and its associated bacterial flora. Concentrations ranged from 100–20,000 $\mu\text{g}/\text{ml}$ for 48–96 hours. The cyst stage survived all concentrations; trophozoites survived 10,000 $\mu\text{g}/\text{ml}$. Thus the antibiotic inhibited bacteria adequately at concentrations that had no effect on the protozoan.⁵⁹ Jacobs in 1947 found that *E. histolytica* trophozoites survived 48 hours in an environment of penicillin. By eliminating bacteria Jacobs maintained *E. histolytica* *in vitro* bacteria-free for 2.5–3.5 months.⁶⁰ In a series of papers reported by Shaffer *et al* on the effects of antibiotics on growth of amoeba, much data was presented. Concentrations of 1,000 $\mu\text{g}/\text{ml}$ penicillin and 1,500 $\mu\text{g}/\text{ml}$ streptomycin result in excellent growth of *E. histolytica* trophozoites. This work was repeated on two strains of *E. histolytica*, and as many as one hundred transplants in the absence of a multiplying bacterial flora were recorded.^{61,62} See Table 6–4.

In a study of the effect of antibiotics and vitamins on *E. histolytica* cultivation, Nelson found that ascorbic acid and calcium pantothenate, when combined with streptomycin, caused death of the amoeba. Evidently a toxic effect was manifested by streptomycin in the presence of these vitamins.⁶³

A new innovation for cultivation of *E. histolytica* without actively growing bacteria was reported in the Japanese literature. Saito pre-conditioned a medium with bacteria, and then added antibiotics to kill the bacteria. In this medium *E. histolytica* grew well and indefinite subcultures were possible.⁶⁴

More recently a quantitative study of amoeba from fecal specimens

TABLE 6-4

RESULTS OF TRANSFER OF *E. HISTOLYTICA* OF THE NRS STRAIN, IN NRS BACTERIA INACTIVATED BY COMBINED HEATING AND ADDITION OF PENICILLIN SODIUM AND STREPTOMYCIN

Transfer	Amoeba growth (48 hrs.)	Bacterial subcultures ¹		Transfer	Amoeba growth (48 hrs.)	Bacterial subcultures (48 hrs.)
		0 hours ²	48 hours ³			
1	+++ ⁴	Neg.	Neg.	16	+++	Neg.
2	+++			17	++	
3	+++			18	++++	
4	+++			19	+++	
5	++			20	+++	
6	++			90	++	
7	++			91	+++	
8	++			92	++	
9	++			94	++	
10	++			95	+++	
11	+++			96	++	
12	+++			97	+++	
13	++			98	+++	
14	+++			99	+++	
15	+++			100	+++	

¹ Fluid thioglycollate medium was used routinely for subculture after transfer 3. Nutrient broth was used in the first 3 transfers.

² Subculture was taken immediately after inoculation with amoebae.

³ Subculture was taken 48 hours after inoculation with amoebae.

⁴ +++ = 25 to 50 motile amoebae trophozoites per low power microscopic field; ++ = 10 to 25 organisms per field.

Shaffer & Frye, 1948, *Am. J. Hyg.* **47**, 214.

showed that 250 µg/ml of streptomycin and penicillin doubled the number of amoeba found by culture as compared with cultures not containing penicillin or streptomycin.⁶⁵

Many other protozoa and related organisms have been cultivated in the presence of antibiotics. Haemoflagellates, *Euglena*, cestodes and nematodes are among those studied.

Seneca cultivated *Trypanosoma cruzii* in bacteria-free culture containing 5,000 µg/ml of penicillin and 5,000 µg/ml of streptomycin. These concentrations of antibiotics had no effect on the vitality and intensity of this protozoan.⁶⁶ Cestodes that were cultivated successfully in the presence of penicillin, streptomycin, and chloromycetin include *Hymenolepis diminutes* and *Hymenolepis nana*. Aureomycin and penicillin proved too toxic, and the penicillin-streptomycin-chloromycetin media allowed yeast contamination, according to Reid *et al.*⁶⁷ In order to obtain bacteria-free cultures of *Euglena*, Pappas and Hoffman had to

use penicillin, dihydrostreptomycin and chlortetracycline. They tried bacitracin and sulfadiazine, but these antibiotics were toxic to the strain tested.⁶⁸

Antibiotics have also been used in media for cultivating the parasites of animals. Recently Delappe used penicillin and streptomycin in a medium for the initial isolation of *Histomonas meleagridis* from turkeys. The results were not clear-cut, in that bacterial inhibition was evident but the culture was not bacteria-free. The use of high concentrations of aureomycin, penicillin, streptomycin and circulin resulted in injury to this protozoan, and consequently bacteria-free cultures were unobtainable.⁶⁹

For many years the protozoologists have been investigating protozoan growth requirements. The foregoing contributions made by the use of antibiotics have served to stimulate and increase the work in this area. A complete review of the overall problem of the growth of protozoa is given in a recent New York Academy of Science publication.⁷⁰

G. VIRUSES AND RICKETTSIA—CULTURE MEDIA USING ANTIBIOTICS

Isolation, cultivation and identification of viruses and rickettsiae are procedures particularly subject to much bacterial and fungal contamination. Since most specimens, i.e., throat washings, sputum, feces, made available for this isolation are originally contaminated with bacteria, the early techniques relied upon filtration of specimens with bacterial filters. The advent of antibiotics stimulated application of antibacterial agents to specimens for isolation of viruses and related organisms. Since antibiotics were effective against bacterial cells but innocuous to other types of cells, they became the agents of choice, first as adjuncts to filtration of specimens, and then later as substitutes for filtration. Still more recently, antibiotics were used to prevent contamination after isolation and cultivation of the virus was in progress. Antibiotics have been incorporated in chick embryo, tissue culture and cell suspensions in which virus was to be maintained.

This work began in the middle 1940's after the remarkable antibacterial capabilities of penicillin had been firmly established. In 1944, in a study of cultivation of typhus rickettsia in yolk sac, Greiff and Pinkerton reported an adverse effect of penicillin. They observed an inhibition of the active growth phase of murine typhus rickettsia in the yolk sac of embryonated eggs.⁷¹ Following this report, there were a series of studies to evaluate possible toxic or injurious effects of penicillin and other agents against tissue cultures, chick embryos and extra-embryonic membranes. This considerable literature has recently been reviewed.⁵

A method for the isolation of influenza virus without filtration was proposed by Burnet and Stone.⁷² They introduced sulfadiazine into the chick embryo, and inoculated it with a mixture of throat washings and penicillin. Successful isolation of influenza types A and B were obtained in this manner. Hirst⁷³ had similar success when he used a somewhat higher level of penicillin, and so did away with the sulfadiazine. An attempt was made to use this procedure in isolating mumps virus directly from the saliva of men by centrifugation and penicillin treatment to obtain a final concentration of 300 units. Isolation was accomplished in 3 of 7 cases, and no injurious effect of the drug was noted.⁷⁴ The availability of streptomycin in 1946⁷⁵ resulted in studies of streptomycin alone and in combination iwth penicillin for virus isolation. Florman *et al* found that streptomycin enabled cultivation of PR8 and other

TABLE 6-5
CULTURES OF MOUSE LUNG

Time of sacrifice after intranasal inoculation	With antibiotics		Without antibiotics	
	Aerobic	Anaerobic	Aerobic	Anaerobic
24 hours	No growth	No growth	50 colonies*	32 colonies
48 hours	4 colonies	No growth	144 colonies	400 colonies

* The colonies consisted of nonhemolytic gram-negative rods, alpha-hemolytic streptococci, and anaerobic streptococci.
McKee and Hale, 1947, *Science* 105, 41-42.

influenza virus strains in the chick embryo.⁷⁶ McKee combined streptomycin (1,000 $\mu\text{g/ml}$) and penicillin (500 $\mu\text{g/ml}$) for isolation of A and B strains of influenza virus. Loss of embryos due to bacterial contamination was lowered and successful virus isolation was made possible.⁷⁷ See Table 6-5. A report that some viruses could not be handled in this manner was published,⁷⁸ indicating that penicillin inhibited psittacosis virus in roller tube tissue culture. Streptomycin, on the other hand, in the same study had no effect on psitacosis virus strain 6BC.

Further attempts to use antibiotics in rickettsial isolation have been made from time to time. The difficulty encountered in the susceptibility, to some degree, of rickettsia to almost every antibiotic. Morgan tested the effect of streptomycin (0.5-2.0 $\mu\text{g/egg}$) on three species of rickettsia. He found streptomycin slowed the growth of all species to some extent.⁷⁹ Thus the application of antibiotics to cultivation of rickettsia has proceeded much more slowly than similar work with the viruses.

Further contributions along these lines have been made by those investigating veterinary virus problems. Several workers have been interested in cultivation of the Newcastle's virus in chick embryo with antibiotic adjuncts. Brandly *et al* demonstrated that 1,000 $\mu\text{g}/\text{ml}$ of penicillin added to 0.1 ml of virus inoculum intended for egg inoculation gave increasingly successful inoculation.⁸⁰

Beaudette and his group used mixtures of penicillin and streptomycin (1,000 μg each/ml) for isolating Newcastle's virus from exudates and tissue suspensions. He maintained bacterial sterility in 99 of 100 samples.⁸¹

A study made to recover Newcastle's virus from the air of poultry houses, with the aid of penicillin and streptomycin in the allantoic fluid, resulted in successful isolation of the virus.⁸² In a study of diarrhea, Hodges tried to isolate a virus. He maintained bacteria-free conditions in 54 out of 55 embryos treated with penicillin and streptomycin.⁸³

Further effects of the use of streptomycin and penicillin in maintaining bacteria-free conditions were apparent from a study with vaccinia virus. Iwasaki added these antibiotics to vaccine lymph tyrode suspension, and inoculated a chick embryo. The vaccinia virus was propagated, and all inoculated eggs were bacteria-free.⁸⁴

Of most recent importance have been attempts to control yeast and fungal contamination of virus cultures. This problem has become more pressing because of the large scale tissue cultures needed for processing various vaccines in quantity. Heretofore tissue culture was carried out on a small scale, and this kind of contaminant was not readily observed.

Wigmore and Henderson, cultivating foot and mouth virus on epithelial tissue culture, added 20 $\mu\text{g}/\text{ml}$ of nystatin and completely controlled yeast contamination.⁸⁵ They had previously used penicillin and streptomycin for bacteria-free conditions.

In a similar work McLimans *et al* reported a very complete analysis of nystatin in the control of fungal and yeast contaminants in tissue culture. Using artificial inocula of *Penicillium*, *Aspergillus*, and *Saccharomyces* in Hela, "L" and chick fibroblast tissue culture, they reported excellent control at approximately 30 $\mu\text{g}/\text{ml}$ culture media. See Table 6-6. The antibiotic showed toxicity for Hela cells at 250 $\mu\text{g}/\text{ml}$, and for "L" and chick fibroblast cultures at 500 $\mu\text{g}/\text{ml}$.⁸⁶

In summarizing the use of antibiotics in isolation and cultivation of viruses, there are certain clear-cut features which determine the choice of antibiotics for these procedures. In spite of the fact that many new antibiotics have come on the scene since penicillin and streptomycin, these two remain the agents of choice in maintaining bacteria-free conditions in viral specimens, chick embryo cultures and tissue cultures.

TABLE 6-6
RELATIVE EFFICACY OF NYSTATIN AS AN ANTIFUNGAL
AGENT EMPLOYED IN TISSUE CULTURES

Experimental inoculum, conidia/ml	Tissue cell line	Minimal inhibitory concentrations, units/ml/7 days		
		<i>Penicillium notatum</i>	<i>Aspergillus niger</i>	<i>Saccharomyces cerevisiae</i>
	Hela cell			
10 ¹		<15.6	31.2	<15.6
10 ²		<15.6	31.2	<15.6
10 ³		31.2	62.5	15.6
10 ⁴	(Human sera)	93.7	62.5	23.5
10 ⁵		1,500	250	31.2
10 ⁶		1,500	500	250
	Chick fibroblast			
10 ¹		15.6	15.6	15.6
10 ²		15.6	46.8	15.6
10 ³		46.8	125	23.5
10 ⁴	(Horse sera)	250	125	31.2
10 ⁵		250	125	31.2
10 ⁶		250	187	375
	"L" cell			
10 ¹		<15.6	23.5	<15.6
10 ²		<15.6	31.2	15.6
10 ³		23.5	62.5	15.6
10 ⁴	(Horse sera)	46.8	125	31.2
10 ⁵		62.5	125	250
10 ⁶		125	375	750

McLimans *et al*, *Antibiotics Annual* 1955-56, 690-696. Med. Encyclopedia Inc., N. Y.

Other antibiotics such as chlormycetin, the tetracyclines and the polypeptide antibiotics have had only limited use for several reasons. The broad spectrum antibiotics are less stable, they combine with animal proteins, and they inhibit the large viruses to a marked degree.⁸⁷ Although, in chick embryo culture there is evidence that eggs contain a factor which inhibits or reverses the breakdown of tetracyclines.⁸⁸ Polypeptide antibiotics are relatively toxic for animal cells, and their antibacterial spectrum is usually limited.⁸⁹

In virus cultivation the two most generally available antifungal antibiotics are cycloheximide and nystatin. The former is quite toxic for animal cell tissue culture, and as a result would have little application for the purpose.⁹⁰ Nystatin is effective in virus cultivation, as is evidenced by the two detailed studies reported above.^{85,86}

Current knowledge, therefore, would seem to require addition of 3

antibiotics to specimens and cultures for virus isolation and growth, these antibiotics being penicillin, streptomycin and nystatin.

H. MISCELLANEOUS CULTURE MEDIA USING ANTIBIOTICS

Modifications of procedures in the bacterial analysis of water, milk and food have been brought about by antibiotic techniques. Rittenberg and Silliker advocated the use of antibiotics in presumptive medium for water analysis. They used penicillin and tyrothricin in lactose broth to suppress undesirable organisms.⁹¹ Spencer in 1952 recommended penicillin and streptomycin to obtain bacteria-free cultures of marine phytoplankton organisms.⁹²

In a study to isolate fungi from a sewage treatment plant, tests were made on media containing, in various relationships, rose bengal dye, streptomycin, chlortetracycline and oxytetracycline. Best results were obtained by dye plus streptomycin.⁹³ Recently in 1956, a review of "standard methods" for water analysis listed antibiotics and related agents in a research study for improved methods of coliform detection.⁹⁴

In a comprehensive review of the detection of coliform bacilli in milk, Olsen has evaluated penicillin, tyrothricin, gramicidin and bacitracin.

TABLE 6-7

CONCENTRATIONS OF CYCLOHEXIMIDE REQUIRED TO SUPPRESS
GROWTH OF VARIOUS ALE AND LAGER YEASTS
IN SOLID SYNTHETIC MEDIUM

Brewery	Yeast type	Concentration, $\mu\text{g/ml}$ of medium						
		0	0.04	0.2	0.5	1.0	2.0	4.0
		Growth, 7 days						
A ₁	Ale	+	+	+	+	—	—	—
A ₂	Ale	+	+	—	—	—	—	—
A ₃	Ale	+	+	—	—	—	—	—
B	Ale	+	+	+	+	+	—	—
C	Ale	+	+	—	—	—	—	—
D	Lager	+	+	—	—	—	—	—
E	Lager	+	+	—	—	—	—	—
F	Lager	+	+	+	—	—	—	—
G ₁	Lager	+	+	+	—	—	—	—
G ₂	Lager	+	+	—	—	—	—	—
H	Lager	+	+	—	—	—	—	—

He prefers EMB agar plus penicillin and regards it as the most satisfactory plating medium that can be used in detecting coliform bacteria.⁹⁵

A number of antibiotics have been studied to establish a synthetic selective medium capable of inhibiting yeasts without affecting the bacteria encountered in brewing, alcoholic fermentation or production of baker's yeast. A medium was developed containing cycloheximide which enabled investigation of the nature and number of viable bacteria in various fermentation materials.⁹⁶ See Table 6-7.

I. SUMMARY

The number of possible extensions of the uses of antibiotics as described above is very large indeed, and depends upon the microorganism under investigation. As new antibiotics emerge, they will undoubtedly be tested for use in selective media.

Obviously, an antibiotic which is to find use in a selective medium for the isolation and cultivation of microbes should comply with a number of criteria, including the following:

1. Stability in the cultivating medium
2. Solubility in the cultivating medium
3. A highly specific antimicrobial spectrum
4. Freedom from toxicity for the organism to be cultivated.

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	Penicillin	Na-oleate	Blood
Pos. for <i>H. inf.</i>	59	55	33
Neg. for <i>H. inf.</i>	60	64	86
	<hr/> 119	<hr/> 119	<hr/> 119

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cycline and chlortetracycline at various concentrations were compared to streptomycin at 30 $\mu\text{g/ml}$ in this medium. The antibiotics most effective were the polycyclines. Chlortetracycline 215 $\mu\text{g/ml}$ inhibited a greater number of bacteria and allowed the growth of more fungal colonies than media containing streptomycin.

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Inoculation of 3 contaminated cultures of *T. vaginalis* into a medium consisting of Locke's solution, goat serum, septamide, and rice starch containing 90 units penicillin/cc. The flagellates grew well and were subcultured to a medium containing 250 units penicillin/cc.

54. Johnson, G., Trussell, M. and John, F., 1945, Isolation of *Trichomonas vaginalis* with penicillin. *Science* **102**, 126-128.

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Cultures of *T. vaginalis* contaminated with gram-positive and gram-negative bacteria were purified by the addition of 25 units/ml of streptomycin after 10 hours exposure at 37°C.

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A brief review of previous cultivation of *T. foetus* was given. This included the isolation of *T. foetus* from bacteria-contaminated material by the use of drugs and mechanical devices (migration, centrifugation, micromanipulation). Described a method of obtaining *T. foetus* in pure culture by the use of media containing 30 units penicillin per ml. Obtained 2 strains of *Trichomonas* free from bacteria by using penicillin.

57. Morgan, B. B., 1946, The use of antibiotics in the purification of *Trichomonas foetus* (protozoa) cultures. *Anat. Rec.* **94**, 437.

Attempted purification of cultures of *T. foetus* by eliminating staphylococci, streptococci, and *E. coli*, *B. subtilis*, and *Corynebacterium* with penicillin and streptomycin. Did not purify from *E. coli* and *B. subtilis* and *Strept. fecalis* with 50,000 units penicillin/ml and 1,000 units streptomycin/ml. 100–500 units/ml penicillin eliminated staphylococci and streptococci. 10,000 units penicillin/ml had no effect on the *Trichomonas*.

58. Williams, L. F. and Plastring, W. N., 1946, Use of antibiotic substances for freeing *Trichomonas foetus* from bacteria. *J. Bact.* **51**, 127.

The use of 100 units each of penicillin and streptomycin combined/ml of medium controlled all bacterial contaminants sufficiently to permit growth of *T. foetus*. Clavacin, gramicidin, and actinomycin in concentrations sufficient to control bacteria were toxic for trichomonas.

59. Balamyth, W. and Wieboldt, M. L., 1946, Action of streptomycin on *Endamoeba histolytica* in vitro. *J. Parasitol.* **32** (Dec. suppl.), 10.

Studies on effect of streptomycin on the NRS "strain" of *E. histolytica* and its associated bacteria flora in culture. 100–20,000/ μ g streptomycin/ml of culture was used for 48–96 hours. Amoebic cysts survived all concentrations. Trophozoites survived 10,000 units/ml for 48 hours although relatively few recovered.

60. Jacobs, L., 1947, The elimination of viable bacteria from cultures of *Endamoeba histolytica* and the subsequent maintenance of such cultures. *Am. J. Hyg.* **46**, 172–176.

Found that *E. histolytica* trophozoites would survive 24–48 hours when transplanted into conditioned medium containing dead bacterial cells and penicillin. Live bacteria inoculated with amoebae were inhibited by penicillin. In 2 instances achieved maintenance of *E. histolytica* in vitro, one for 3.5 months, the other for greater than 2.5 months. Freed unibacterial cultures of *E. histolytica* from *Cl. perfringens* by means of penicillin.

61. Shaffer, J. C. and Frye, W. W., 1948, Studies on growth requirements of *Endamoeba histolytica*. I. Maintenance of a strain of *E. histolytica* through one hundred transplants in the absence of an actively multiplying bacterial flora. *Am. J. Hyg.* **47**, 214.

Series of experiments to study effects of antibiotics on amoebal and on bacterial flora accompanying the amoebae. The concentration of penicillin and streptomycin which inhibited bacterial multiplying had little if any effect on multiplication of *E. histolytica*—used 950–1,900 oxford units penicillin/ml and 1,500 μ g streptomycin/ml.

62. Shaffer, J. G., Ryden, F. W. and Frye, W. W., 1948, Studies on the growth requirements of *Endamoeba histolytica*. III. The growth and multiplication of two strains of *E. histolytica* in a transparent medium without

the addition of rice flour or other particulate matter and without demonstrable bacterial growth. *Am. J. Hyg.* **47**, 345.

Trophozoites of *E. histolytica* inoculated into antibiotic media. Excellent growth and multiplication occurred in 48 hours, in tubes incubated anaerobically. When plating from stools, the concentrations of penicillin G was increased to 1,000 $\mu\text{g/ml}$ and streptomycin to 1,500 $\mu\text{g/ml}$.

63. Nelson, Clifford E., 1949, Some observations on vitamins and antibiotics in the cultivation of *Endamoeba histolytica*. *Jour. Parasitol.* **35** (6), 17.

Egg-yolk-extract medium cultures+streptomycin caused death of the amoebae. Addition of ascorbic acid and calcium panthothenate to above antibiotic containing medium brought no success.

64. Saito, M., 1953, Cultivation of *Endamoeba histolytica* without actively growing bacteria; cultivation in preconditioned medium with antibiotics added. *Jkitasato Arch. Exper. Med.* **25**, 245-252.

"By preconditioning the media with the entire bacterial flora of the stock culture and by the addition of antibiotics, *E. histolytica* grew without any actively growing bacterial associates, and indefinite subcultures were possible...."

65. Norman, L. and Brooke, M. M. 1955, The use of penicillin and streptomycin in routine cultivation of amoeba from fecal specimens. *Am. and Trop. Med. Hyg.* **4**, 472.

The addition of 250 units/ml of overlay each of penicillin sodium and streptomycin sulfate doubled the number of amoebae found by culture as compared with cultures not containing penicillin and streptomycin. The antibiotics inhibited growth of *Blastocystis hominis*. Antibiotic cultivation, however, did not help those cultures from old specimens, but did help where fresh specimens could be cultured.

66. Seneca, H., Henderson, E. and Harvey, M., 1949, Purification of hemoflagellate cultures with antibiotics. *Am. J. Trop. Med.* **29**, 41.

Neither penicillin in 1,000 units and 5,000 units/5 cc supernatant overlay, nor similar amounts of streptomycin, nor 5,000 units each of penicillin and streptomycin together, had any effect on the vitality and the intensity of the growth of *Leishmania donovani* and *Trypanosome cruzi*. For the purification of contaminated cultures of hemoflagellates, 5,000 units of penicillin and 5,000 units of streptomycin added to such cultures in 5 cc of saline ordinarily kills all contaminants in the 2nd generation.

67. Reid, W. M. and Boles, J. I., 1949, Antibiotics as bacteriostatic agents for the cultivation of cestodes *in vitro*. *Jour. Parasitol.* **35** (6), 37.

Various antibiotics used to prevent overgrowth of bacteria in culture for *in vitro* cultivation of *Hymenolepis diminuta* and *H. nana*. Best results obtained with a maximum survival time of 10 days by various combinations of penicillin (1,852 units/cc), streptomycin (1,000 $\mu\text{g/cc}$) and chloromycetin (800 $\mu\text{g/cc}$). Medium—Locke's solution and other nutrients. Aureomycin (500 $\mu\text{g/cc}$)+penicillin was too toxic, got effective bacterial inhibition but much yeast contamination with the penicillin, streptomycin, and chloromycetin media.

68. Pappas, G. and Hoffmann, H., 1952, The use of antibiotics for obtaining bacterial free cultures of *Euglena*. *Ohio Jour. of Science* **52** (2), 102-105.

Bacterial free cultures were obtained only when a combination of penicillin (2,000 $\mu\text{g/ml}$), dihydrostreptomycin (1,000 $\mu\text{g/ml}$) and aureomycin (400 $\mu\text{g/ml}$) was used. Bacitracin and sulfadiazine were toxic to the strain tested.

69. Delappe, Irving Pierce, 1953, Studies on *Histomonas meleagridis*. 1. Use of antibiotics to facilitate *in vitro* isolation. *Exptl. Parasitology* 2 (1), 79-86.

The incorporation of penicillin and streptomycin (100 or 500 $\mu\text{g/ml}$) either singly or conjointly in Laidlaw's medium facilitated the initial isolation of the organism from the turkey. The *in vitro* survival time of organisms was prolonged. Cultures not bacteria free but evidence of bacteria inhibition. Subsequent attempts to obtain bacteria free cultures without injuring protozoa utilizing higher concentrations of aureomycin, penicillin, streptomycin, and circulin, were abortive.

70. Growth of Protozoa, 1953, *Ann. N. Y. Acad. Sci.* 56, 815-1094.

A thorough review by active investigators of isolation, cultivation, ecology and metabolism of protozoa.

71. Grieff, D. and Pinkerton, H., 1944, Inhibition of growth of typhus rickettsiae in the yolk sac by penicillin. *Proc. Soc. Exp. Biol. and Med.* 55, 116-119.

The active growth phase of murine typhus rickettsiae in yolk sac of embryonating eggs was inhibited by introduction of penicillin.

72. Burnet, F. M. and Stone, J. D., 1945, A method for the isolation of influenza virus from throat washings without filtration. *Australian J. Exp. Biol. and Med. Sci.* 23, 161-163.

Isolation of influenza virus (A and B) from throat washings injected 0.1 ml of 5% sulfadiazine into amniotic sac of 13 day chick embryos followed by 0.1 ml of throat washing penicillin mixture containing 10 units penicillin. No injurious effects on virus or embryo.

73. Hirst, G. K., 1945, Direct isolation of influenza virus in chick embryos. *Proc. Soc. Exp. Biol. and Med.* 58, 55-157.

Throat washings from influenzae A patients and 25-125 units penicillin inoculated into amniotic sac proved to be highly satisfactory method of detecting influenza A virus and was most sensitive of methods known.

74. Beveridge, W. I. B., Lind, P. E. and Anderson, S. G., 1946, Mumps 1. Isolation and cultivation of the virus in the chick embryo. *Australian Jour. Exp. Biol. Med. Sci.* 24, 15-19.

"...attempted direct isolation of mumps virus from saliva of man by first injecting the yolk sac of 5-6 day embryonating eggs with 0.4 ml of 5% sulfadiazine followed by introductions of centrifuged saliva that had been treated with penicillin to make a final concentration of 300 units. Isolation of virus accomplished in three of seven cases, and no injurious effect of drugs was noted.

75. Schutz, A., Bugie, E. and Waksman, S. A., 1944, Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. *Proc. Soc. Exp. Biol. and Med.* 55, 66-69.

The original isolation of streptomycin reporting its characteristics and properties.

76. Florman, A. L., Weiss, A. B. and Council, F. E., 1946, Effect of large doses of streptomycin and influenza viruses on chick embryos. *Proc. Soc. Exp. Biol. and Med.* **61**, 16-18.

Streptomycin did not inhibit the growth of PR8, Olsen and Lee, strains of influenza virus injected into allantoic chamber of 11 to 13 day embryonating chicken eggs.

77. McKee, A. P. and Hale, W. M., 1947, Streptomycin as an aid in isolating influenza virus. *Science* **105**, 41-42.

Influenzae A and *B* infected nose and throat washings and mouse lungs were treated with a combination of 1,000 units/ml of streptomycin and 500 units/ml of penicillin. Loss of embryos due to bacteria contamination was considerably lowered with these concentrations of antibiotic and there was no apparent inhibition of growth of *A* and *B influenzae* virus.

78. Early, R. L. and Morgan, H. R., 1946, Studies on the chemotherapy of viruses in the *Psittacosis-Lymphogranuloma venereum* group. *Jour. Immunol.* **53**, 151-155.

Psittacosis 6 BC inhibited from growing in roller tube cultures by 5 units penicillin/ml as indicated by a rise in virus titer after penicillin treatment of culture was removed. Penicillin inhibited virus 6 BC in eggs at 150-250 μ g/egg. Streptomycin 5 units/ml of tissue culture fluid or in 250 unit doses injected into eggs had no effect on psittacosis 6 BC. Na sulfadiazine had a marked inhibitory action on the growth of psittacosis virus in eggs.

79. Morgan, H. R., Stevens, D. A. and Synder, J. C., 1947, Effect of Streptomycin on growth of Rickettsiae in eggs. *Proc. Soc. Exp. Biol. and Med.* **64**, 342-345.

Streptomycin 0.5-2.0 mg/egg or PABA 5.5 and 11 mg was injected into the yolk sac of 7 day eggs 2 hours prior to infection with *R. mooseri* (Wilmington strain), *R. prowazek* (Breinl strain) or *R. orientalis* (Karp strain). PABA inhibited growth of all; *R. prowazeki* was most sensitive.

80. Brandly, C. A., Moses, H. C., Jones, E. E. and Junghen, E. L., 1946, Isolation and identification of N.D. virus. *Am. Jour. Vet. Res.* **7**, 289-306.

A comprehensive review of the isolation and identification of Newcastle Disease virus. Advocates penicillin treatment of specimen at level of 500-1,000 units/ml and several hours incubation at 37°C for bactericidal effect. The antibiotic did not alter the titer of virus but did eliminate bacterial contaminants.

81. Beaudette, F. R., Vivins, J. A. and Miller, B. R., 1948, Use of antibiotic agents for bacterial sterilization of respiratory exudates from naturally infected cases of Newcastle Disease. *Am. Jour. Vet. Res.* **9**, 97-101.

Mixtures of penicillin and streptomycin, 1,000 units each per ml, used for isolating Newcastle virus from exudates and tissue suspensions, appeared to have no more harmful effect on the recovery of virus than filtration.

82. Delay, P. D., DeOme, K. B. and Bankowski, R. A., 1948, Recovery of Pneumoencephalitis (Newcastle) virus from the air of poultry houses containing infected birds. *Science* **107**, 474-475.

Streptomycin and penicillin used in normal allantoic fluid that had been used to wash air from a poultry house harboring chickens infected with Newcastle Disease. 10,000 units penicillin and 24,000 μg streptomycin/ml allantoic fluid. Successfully isolated the virus from the air.

83. Hodges, J. H., 1946, Effect on chick embryo of the simultaneous inoculation of stool, streptomycin and penicillin. *Science* **104**, 460-461.

Thirteen-day-old chick embryos were inoculated *via* amnion with stool specimen and penicillin-streptomycin (25-200 μg /.05 ml-5 mg/.05 ml) For combating bacterial growth, the combination is effective; either antibiotic alone is inferior.

84. Iwasaki, Hirotugu, 1951, On the cultivation of vaccinia virus using the embryonated hen's egg (In Japanese). *Virus* **1**, 211-214.

When 5,000 units of streptomycin and 500 units of penicillin G were added to 1 ml of vaccine lymph Tyrode suspension and added to allantoic fluid of chick embryo, the vaccine virus could be propagated and all inoculated eggs were free from bacilli.

85. Wigmore, J. O. and W. M. Henderson, 1955, Control of yeast contamination by Mycostatin in cultures of the virus of foot-and-mouth disease. *Nature* **176**, 516.

Epithelial tissue of cattle tongue is used to culture the virus. Effective control of bacterial contamination can be obtained by use of penicillin and streptomycin. The addition of 20 units mycostatin/ml of virus culture has completely controlled yeast contamination. Use of fourfold increases in concentration of mycostatin, no decrease in virus. Multiplication was observed until 320 units/ml had been exceeded.

86. McLimans, W. F., Bonissol, C., Davis, E. V. and Rake, G., 1955-56, Nystatin, an antibiotic useful for the control of fungal and yeast contaminants in tissue culture. *Antibiotics Annual*, 690-696. Med. Encyclopedia Inc., N. Y.

Nystatin at a concentration of 31.2 units/ml of tissue culture media effectively inhibited growth of *P. notatum*, *A. niger*, and *S. cerevisiae* when not more than 100 mold spore or yeast cells were inoculated into actively growing tissue cultures of HaLa, "L" and chick fibroblast cultures. It proved to be toxic for HeLa cells when used levels of 250 units/ml or higher and for "L" and chick fibroblast cultures at 500 units/ml.

87. Woodward, T. E. and Smadel, J. E., 1954, Virus and Rickettsial Diseases in: Antibiotics and Antibiotic Therapy. Edited by Welch, H. Medical Encyclopedia Publisher (N. Y.), pp. 547-572.

A clinical review of antibiotics in treatment of viral and rickettsial diseases. Broad spectrum antibiotics effect "large viruses" only.

88. Womack, C. R., Kass, E. H., Wells, B. E. and Finland, M., 1949, A substance in egg yolk which inhibits deterioration of aureomycin activity. *Proc. Soc. Exper. Biol. and Med.* **72**, 706-708.

Evidence is shown for a factor(s) found in egg yolk which inhibits the breakdown of aureomycin normally occurring at 37°C incubation. This factor is stable to 100°C for one hour and has been autoclaved without loss of activity.

89. Barton, A. L., 1950, Handbook of Antibiotics. Reinhold Publishing Corp. (N.Y.C.).

A description of chemical, physical and biological properties of antibiotics. For the polypeptide antibiotics it describes their toxic effects and narrow antibacterial spectrum.

90. Metzger, J. F., Fusillo, M. H., Cornman, I. and Kuhns, D. M., Antibiotics in tissue culture. *J. Exptl. Cell Research* **6**, 337-344.

This study reviews the literature and reports additional experimental work on toxicity of antibiotics for tissues in culture. Aureomycin and Actidione produce cytologic changes at low levels. At high concentrations many antibiotics inhibit growth of cells completely.

91. Rittenberg, S. C. and Silliker, J. H., 1949, Use of antibiotics in presumptive medium for water analysis. *Amer. Jour. Publ. Health* **39** (12), 1,553-1,560.

The addition of antibiotic substances to lactose broth has been considered as a possible means in increasing the efficacy of the presumptive medium in H₂O analysis. Sensitivity of coliforms, aerobacilli, enterococci, and *Cl. welchii* to penicillin, tyrothricin, and streptomycin was determined. With pure cultures appropriate concentrations of penicillin or tyrothricin could be chosen so as to suppress the undesired organisms without appreciable inhibition of coliforms.

92. Spencer, C. P., 1952, The use of antibiotics for isolating bacteria-free cultures of marine phytoplankton organisms. *Jour. Marine Biol. Assoc. United Kingdom* **31** (1), 97-106.

A method is described for obtaining bacteria-free cultures of certain marine phytoplankton organisms. The method depends upon the selective bacteriostatic action of penicillin and streptomycin. The possibility of applying the method to other organisms has been investigated and the results suggest that, in the case of many of the more nutritionally exacting algae, control of associated bacteria ultimately limits algae growth.

93. Cooke, W. B., 1954, Use in media for isolation of fungi from polluted water. *Antibiotics and Chemother.* **4**, 657.

Isolated fungi from sewage treatment plant to determine mold population in various stages of treatment. Compared rose bengal and phloxine for bacteria inhibition. Compared antibiotics streptomycin sulfate-Aureomycin, terramycin, and chloramphenicol in media containing rose bengal. Results: Media-dye-aureomycin or terramycin more effective in reduction of bacteria counts than media-dye or media-dye-streptomycin or chloramphenicol. Streptomycin better than chloramphenicol. Author suggests use of streptomycin rather than aureomycin or terramycin because of its stability.

94. Neumann, H. G., 1956, Possibilities of newer bacteriological technique. *Jour. Amer. Water Works Assoc.* **42** (1), 57-65.

The test for bacteria of coliform group in H₂O as described in "Standard Methods" has recently undergone some modifications in the interest of flexibility and brevity. Antibiotics and sulfanamides have been used successfully in some cases to improve the coliform method through elective action.

95. Olsen, E. M., 1952, On coliform bacteria in milk. With special reference to the detection. *Den Kgl. Veteriner og Landhøjskols*, p. 184.

Prefers E:M:B: agar-penicillin and regards it as the most satisfactory plating medium that can be used in detecting coliform bacteria. His tests indicate enough value in testing of raw milk for the presence of coliform bacteria to justify using such procedures in connection with plating procedures for determining total count of bacteria.

96. Green, Samuel R. and Gray, P. P., 1951, A differential procedure for bacteriological studies useful in the fermentation industry. *Arch. Biochem. & Biophys.* **32** (1), 59-69.

A synthetic differential medium was developed containing antibiotics capable of inhibiting the growth of yeast without effect on bacteria usually encountered in industrial fermentations, in particular in brewing, alcohol fermentation and production of baker's yeast. The differential medium and technique represent an excellent method for investigating the nature and number of viable bacteria in various fermentation materials.

CHAPTER VII

THE PUBLIC HEALTH SIGNIFICANCE OF NON-MEDICAL USES OF ANTIBIOTICS

BY JOHN T. LOGUE

The results obtained up to the present time by research on the numerous non-therapeutic uses of antibiotics have been reviewed exhaustively in earlier chapters of this book. To appreciate fully the possible public health significance of the non-medical uses of antibiotics, one must realize that up to now such uses have been limited and strictly controlled, both by law and by the care which scientific investigators have exercised in handling antibiotic preparations, in the course of their search for areas in which antibiotics may be of value. Yet, despite these precautions and controls, the question of hazards to public health has arisen.

Since 1941 the medical use of antibiotics has increased so much that there are few people who have not received at least one of these agents in the treatment of disease. Spectacular success has attended the use of antibiotics in the control of many human infectious diseases which, by earlier therapeutic methods, had been serious and often fatal. These favorable results encouraged the widespread and indiscriminate use of antibiotics in the treatment of other human diseases where little promise of success could be anticipated. This frequent, extensive and often unnecessary use of antibiotics has created a considerable reservoir of sensitized individuals who can react violently on exposure even to minute amounts of an antibiotic. Clinical evidence of serious toxic reactions, and often of fatal allergic reactions to therapeutic dosages of all the commonly-used antibiotics is extensive. More recently, the rapid appearance and spread of antibiotic-resistant bacteria give warning that many new problems in the incidence and control of human disease may well appear in the future.

These untoward and undesirable side effects have been caused by widespread therapeutic use of all the commonly-used antibiotics, some of which are most useful in food production, food processing and food preservation. Specifically, penicillin, streptomycin and the tetracyclines (such as chlortetracycline and oxytetracycline) are the antibiotics whose non-medical use may well produce an increased food supply. What will happen when foods containing antibiotics residues are ingested or handled by the large reservoir of antibiotic-sensitive individuals?

One highly important aspect of this question is the fortunate fact that the antibiotic residues determined in foods ready for consumption are far smaller than the therapeutic doses of the parent antibiotics. Furthermore, these residues, because of their instability, may well not exhibit the deleterious action in the host which the parent antibiotic would have.

Antibiotics and their residues find their way into foods for human consumption through several well-recognized channels. Antibiotics are used as feed-supplements in the nutrition of cattle, swine and poultry; they are used in the treatment of animal diseases; and their use is envisioned in the preservation of food. Through these channels, antibiotics may exist in foods at the time of consumption—not only in meat, fish and poultry, but also in animal products, such as eggs, milk, butter and cheese.

Moreover, the hazard is not limited to the ingestion of foods containing antibiotics or their residues. In fact, the most serious potential public health hazard arising from extensive non-therapeutic use of antibiotics is the exposure of the sensitized individuals among those who handle the high concentrations of antibiotics used in plant disease control and food processing. We cannot overlook the tendency of certain antibiotics to induce resistant bacteria. This consideration is important from the ecological viewpoint as well as that of public health.

A final consideration is the possibility that cumulative toxic effects may conceivably result from long-term intake of low-level antibiotic residues.¹

In the following sections of this chapter, this overall public health problem is approached from the standpoint of the various non-medical uses of antibiotics.

A. SOURCES OF ANTIBIOTIC RESIDUES

1. ANTIBIOTICS AS FOOD PRESERVATIVES

The use of antibiotic preparations in the preservation of foods has been exhaustively explored, and much of the evidence indicates that antibiotic preparations can be used successfully and economically to decrease immediate spoilage rates of perishable foods such as vegetables, poultry, red meats, and fish (see Chapter 5). In these times of world-wide food shortages and increasing population, serious consideration must be given to any method that shows the promise of increasing available food supplies for a hungry world. It has also been pointed out that the most successful experiments in the use of antibiotics as food preservatives have been those in which the highest standards of sanitation and refrigeration were used in conjunction with the antibiotic treatment.

The antibiotics recommended for use as food preservatives owe their effectiveness to the exercise of a bacteriostatic function. Antibiotics inhibit bacterial growth of the sensitive bacteria, but do not kill the organisms. It follows that antibiotics function as food preservatives only as long as they are present in or on foods in levels high enough to suppress bacterial growth of the sensitive organisms. Experience has shown that this is indeed the case in food preservation; however, rapid multiplication of non-sensitive organisms can also occur. Moreover, the emergence of antibiotic resistance in organisms that previously were sensitive to the antibiotic occurs when the antibiotic preparations are used for too long a time. In the preservation of fresh-cut vegetables, and the prolongation of shelf-life of vegetables, streptomycin has proved thus far to be the most effective antibiotic for commercial use. Multiple experiments with many antibiotic preparations over the years, in an effort to decrease bacterial spoilage of fish and meats, have shown that chlortetracycline or oxytetracycline have given consistently better results than other antibiotics now in common use.

It follows that the residues of these antibiotics may cause undesirable side effects, either by the exposure of sensitized individuals who handle large quantities of these antibiotics in food preservation methods, or by the continued consumption of the antibiotic residues by the public at large.

TABLE 7-1

PENETRATION OF CHLORTETRACYCLINE INTO GRAY COD STORED IN SEA WATER CONTAINING 2.8 MCG/ML OF THE ANTIBIOTIC **

	Section of fish *	Chlortetracycline in mcg/g after days						
		0	1	2	4	5	6	7
Ordinary ice	V + S	0		0		0		0
	V - S	0		0		0		0
	T + S	0		0		0		0
	T - S	0		0		0		0
Chlortetracycline ice	V + S		1.5	3.0	1.5		2.16	3.0
	V - S		0	1.23	1.23		1.26	1.14
	T + S		1.2	1.23	1.5		1.26	2.55
	T - S		0	0	0		0	0

* V + S—Visceral cavity wall plus skin.
V - S—Visceral cavity wall minus skin.
T + S—Tail end of fish plus skin.
T - S—Tail end of fish minus skin.

** Tarr, H. L. A.².

TABLE 7-2

INACTIVATION OF CHLORTETRACYCLINE ON HEATING FISH FLESH CONTAINING 6.5 AND 16.0 MCG/G OF THE ANTIBIOTIC *

		Time required to attain temperature*	CTC content (mcg/g)	Time required to raise and maintain temperature 5'	CTC content (mcg/g) after holding 5' at temp. given
6.5 mcg/g CTC...	60..	5' 40"	1.4	11' 45"	1.4
		6' 10"	1.2	11' 0"	1.8
	82..	5' 29"	1.4	11' 35"	1.8
		8' 0"	1.8	15' 0"	1.2
	99..	5' 8"	<0.3	10' 0"	<0.3
		4' 50"	0.8	10' 30"	<0.3
16.0 mcg/g CTC...	60..	8' 45"	13.2	12' 35"	2.8
		8' 45"	11.2	12' 35"	2.8
		8' 45"	11.2	12' 35"	2.8
	82..	9' 27"	6.6	16' 0"	2.0
		9' 27"	5.4	16' 0"	3.0
		9' 27"	4.8	16' 0"	2.4
	99..	14' 0"	2.4	10' 35"	0.98
		14' 0"	2.4	10' 35"	1.2
		14' 0"	2.4	10' 35"	0.92

* Tarr, H. L. A.²

For antibiotic residues to be of any significance to a food consumer they must be present in detectable amounts when the food is consumed. The concentrations of chlortetracycline used throughout the many experiments conducted by Tarr and his associates² at the Pacific Fisheries Experimental Station on the preservation of fish by storage in chlortetracycline ice, have been extremely small and amount to a single, and rather small normal daily oral dose in, for example, one ton of ice. As shown in these experiments, only a fraction of the amount used is ever likely to enter the fish, and it is extremely doubtful if the average fish treated with chlortetracycline ice would have more than .2 or even .1 ppm of the antibiotic in its flesh (see Table 7-1).

In fact the amount is so small that it is only rarely that any antibiotic can be detected by the pad plate assay procedure, and that only after fairly prolonged storage in ice. Since fish are almost invariably cooked prior to use, it is extremely doubtful whether it would ever be possible to detect any chlortetracycline in their flesh as consumed even if they

had been stored for considerable periods in chlortetracycline ice (see Table 7-2).

Poultry has always presented a serious problem. Zeigler and Stadelman³ have shown that poultry which has been dressed, washed, cooled, and dipped for ten minutes in chlortetracycline solution containing ten to forty ppm keeps longer than control birds, without bacterial spoilage. Kohler⁴ has shown that chlortetracycline is the most effective antibiotic for the purpose of controlling the growth of organisms isolated from spoiled poultry when compared with the following antibiotics: chloramphenicol, erythromycin, procaine penicillin, neomycin, streptomycin, magnamycin, polymyxin B, bacitracin and cycloheximide. Data for oxytetracycline in almost all studies give results directly comparable with chlortetracycline. These observations have been further extended by Gibson.⁵ In further experiments, Kohler⁴ has shown that the shelf life of poultry treated with chlortetracycline is almost double that of untreated controls. In addition, and most important from the public health standpoint, Kohler⁴ and Durbin⁶ have shown that the chlortetracycline residues in poultry flesh are destroyed by cooking. As a result of these investigations, and related studies on oxytetracycline outlined in Chapter 5, these two antibiotics have found commercial application in poultry processing.

Durbin and his associates⁶ have further shown that no antibiotic (chlortetracycline) could be detected in eggs from hens fed 3, 10, 50, or 100 ppm of the drug. At 200 ppm, chlortetracycline was consistently present in the eggs while the hens were consuming the antibiotic, but as soon as it was removed, the eggs laid did not contain a detectable amount (see Table 7-3).

Eggs from hens on a diet of 200 ppm chlortetracycline were hard-boiled for ten minutes. Nineteen of twenty eggs were negative for the drug after cooking, when compared with seven of fifteen positive tests for unboiled eggs collected from the same hens during the same time period. In eggs from hens fed a diet containing 10,000 ppm of chlortetracycline, only 50% of the eggs contained chlortetracycline after boiling for ten minutes. It is thus apparent that eggs from poultry flocks fed high levels of chlortetracycline could conceivably have detectable amounts of the drug after cooking, but that the levels now commonly used as growth adjuncts in poultry and egg-laying flocks would not be a source of antibiotic exposure to the consumer.

Goldberg *et al.*⁷ have shown that chlortetracycline enhances the keeping quantities of hamburger meat both in the refrigerated and unrefrigerated state. These observations have been extended by Weiser and his associates⁸ by infusing whole animals with chlortetracycline

TABLE 7-3
CHLORTETRACYCLINE IN HENS' EGGS

Chlortetracycline ppm (g per ton) of feed	Weeks on feed			Weeks off feed		
	1	2	3	1	2	3
	Chlortetracycline, mcg/g. of eggs					
	Ave.	Range	Ave.	Range	Ave.	Range
50 ppm	0	—	0	—	0	—
100 ppm	0	—	0	—	0	—
200 ppm	.10	.05-.13	.06	.05-.10	.03	.04-.72
2,000 ppm	.56	.46-.71	.48	.40-.62	.43	.36-.64
10,000 ppm	.13	.09-.16	.17	.14-.19	.44	.21-.67
20,000 ppm	.31	.14-.64	No eggs laid		2.56	—
					4.0	—
					.19	.28-.29
					.02	.04-.04
					.02	.06-.12
					.07	.04-.13
					.03	.03-.05*

The 0 (control), 3 and 10 ppm were negative throughout the experiment.

* Neg. 4th wk. Durbin *et al.*⁶

before dressing out. These experiments indicate that chlortetracycline does delay deep spoilage of meat in carcasses where refrigeration is delayed. Chlortetracycline residue levels of 2 ppm were measured in the meat at slaughter. They showed further that detectable levels of chlortetracycline decreased rapidly in the infused meat, both in those meat samples

TABLE 7-4
THE EFFECT OF COOKING ON OXYTETRACYCLINE
RESIDUES IN BEEF

Beef sample	Injected antemortem	Amount of 20% OTC injected	OTC ppm/gm (raw)	OTC ppm/gm cooked 140°F (rare beef)	OTC ppm/gm cooked 160°F (medium well)
1	Control		<.06	<.06	<.06
2	1 hr.	3 mg/lb	.19	.13	<.06
3	1 hr.	3 mg/lb	.15	.13	<.06
4	30 min.	3 mg/lb	<.06	<.06	<.06
5	30 min.	3 mg/lb	<.06	<.06	<.06
6	1 hr.	3 mg/lb	.10	<.06	<.06
7	1 hr.	3 mg/lb	.11	<.06	<.06
8	30 min.	3 mg/lb	<.06	<.06	<.06
9	30 min.	3 mg/lb	<.06	<.06	<.06
10	Control		<.06	<.06	<.06
11	Control		<.06	<.06	<.06
12	1 hr.	4 mg/lb	<.06	<.06	<.06
13	1 hr.	4 mg/lb	<.06	<.06	<.06
14	30 min.	4 mg/lb	.15	.10	<.06
15	30 min.	4 mg/lb	.14	.11	<.06
16	Control		<.06	<.06	<.06
17	1 hr.	4 mg/lb	<.06	<.06	<.06
18	1 hr.	4 mg/lb	.09	<.06	<.06
19	2 hr.	4 mg/lb	.26	.21	<.06
20	2 hr.	4 mg/lb	.19	.22	<.06
21	4 hr.	4 mg/lb	.18	.14	<.06
22	4 hr.	4 mg/lb	.15	.19	<.06
23	30 min.	4 mg/lb	<.06	<.06	<.06
24	30 min.	4 mg/lb	<.06	<.06	<.06
25	6 hr.	4 mg/lb	.15	.16	<.06

that were refrigerated immediately and in those meat samples where refrigeration was delayed. After five days chlortetracycline was present in about 1.0 ppm in the chilled meat. In meat samples where refrigeration was delayed, there were no detectable levels of chlortetracycline at five days.

In Table 7-4, one can see the effects of cooking on the oxytetracycline residues in paired beef samples. Levels as high as .18 ppm in raw beef are decreased to .14 ppm when cooked at 140°F (rare beef) and to less than .06 ppm when cooked at 160°F (medium well done). In all samples handled in similar fashion a similar effect is demonstrated. This study was carried out by the meat laboratory (H. D. Naumann and R. B. Sleeth) of the University of Missouri and has not previously been reported.

Becker⁹ has shown that low-level treatments with chlortetracycline, oxytetracycline and streptomycin reduced the amount of decay and improved the appearance of prepackaged spinach. These treatments significantly reduced the size of the bacterial populations present. When spinach treated with oxytetracycline or chlortetracycline was boiled for three minutes, almost all of the bacteriological activity was lost. Streptomycin, on the other hand, persists in spinach in levels of 2.05 mg/ml despite cooking. In further experiments, Goodman¹⁰ has demonstrated that oxytetracycline is heat labile in potato tissue, and that oxytetracycline gradually loses its bacteriological effect in stored cabbage. Streptomycin when used for a similar purpose has been shown to be exceedingly stable in apple and potato tissue¹¹ as well as resistant to destruction by heat.¹²

From the above discussion, it is obvious that detectable levels of bacteriologically-active chlortetracycline and oxytetracycline residues may be found in poultry, eggs, beef, fish and spinach when they are delivered to the consumer. It is equally obvious that cooking destroys the major part of the bacteriological capability of these low residues. If it can be shown that the heated breadkown products of these antibiotics have no deleterious effects on humans after consumption, there would seem to be little reason not to use these antibiotics as food preservatives in the above-mentioned manner if the practical advantage of such use can be demonstrated.

The use of streptomycin in harvested vegetables, on the other hand, because of its extreme stability and high sensitizing potential must be regarded as a more dangerous method of food preservation.

2. ANTIBIOTICS IN PLANT DISEASE CONTROL

Since 1951, when Murneek¹³ first demonstrated that low-potency streptomycin sprays resulted in a 60% reduction in the incidence of the

TABLE 7-5
RESIDUAL ANTIBIOTIC ACTIVITY ON APPLE LEAVES *

Days	<i>E. amylovora</i> (E-4) mcg/ml	<i>K. pneumoniae</i> (102) mcg/ml
2	37.1†	9.4
4	38.6	15.0
6	14.7	13.6
8	20.0	8.6
13	26.0	14.5
17	17.8	6.6
19	25.0	3.9
27	16.2	2.7

† Treatment: Apple shoots sprayed once with the antibiotic combination at 100 mcg/ml. Morgan *et al.*¹⁴

disease, fireblight, in apple trees, there have been intensive studies on the use of antibiotic preparations to prevent, control, or eradicate existing bacterial diseases in a wide variety of food producing plants. The most gratifying results have been obtained from spraying large acreages of food producing plants with various solutions of streptomycin and oxytetracycline. It is, therefore, from these two antibiotics that one would expect to discover adverse side effects, either among the workers who handle these materials in large quantities, or perhaps in the food consumer who might ingest foods containing considerable quantities of the antibiotic residue, or who might even develop difficulty from direct contact with the antibiotic residue remaining on or within the plant product.

Using a bioassay for the detection of residue of the antibiotic combination of streptomycin and oxytetracycline, Morgan¹⁴ has demonstrated

TABLE 7-6
RESIDUAL ANTIBIOTIC ACTIVITY IN MATURE APPLE FRUIT

Treatment	Sample	Activity in mcg/ml	
		Neutralized	Non-neutralized
4 sprays (100 mcg/ml)*	Flesh	0.0†	30.0
4 sprays (100 mcg/ml)	Skin	0.0	0.0
Control	Flesh	0.0	30.0
Control	Skin	0.0	0.0

* Trees sprayed four times with the antibiotic combination at 4-day intervals, April 19 to May 3. Mature fruit harvested September 16.

† Sensitivity of assay 0.3 mcg/ml; assay organism *Erwinia amylovora* (E-4). Morgan *et al.*¹⁴

TABLE 7-7
STREPTOMYCIN ASSAYS

Treatment number	Average* of three replicates (parts per million)	
	1st week	2nd week
I	<0.09	<0.09
II	<0.09	<0.09
III	<0.09	<0.09
IV	<0.09	<0.09
V	<0.09	<0.09

* Each replicate assayed <0.09. Carroll *et al.*¹⁵

residual antibiotic activity on apple leaves as long as 27 days after a single spraying of an apple tree with streptomycin-oxytetracycline solution in concentration of 100 mg/ml (see Table 7-5). Assay of mature apple fruit, sprayed four times at four-day intervals with 100 mg/ml of antibiotic combination four and one-half months prior to harvest, failed to detect any antimicrobial activity that could be ascribed to the antibiotic sprays (see Table 7-6).

Carroll and co-workers,¹⁵ using a different microbiological assay technique, could detect no detectable residue of streptomycin or oxytetracycline in tomatoes assayed one week after the last spray. The tomato plants had been sprayed frequently in the field for seven weeks (see Table 7-7 and Table 7-8).

These findings indicate that spraying of fruit and vegetables during their early growth will not result in detectable antibiotic residue levels in the mature fruit or vegetable. These findings should be confirmed and extended to cover all consumer goods from plants thus treated.

TABLE 7-8
OXYTETRACYCLINE ASSAYS

Treatment number	Average of three replicates (parts per million)		
	1st week	2nd week	3rd week*
I	0.016	0.017	<0.006
II	0.022	0.031	<0.006
III	0.032	0.058	<0.006
IV	0.023	0.046	<0.006
V	0.033	0.022	<0.006

* In this series, each replicate assayed <0.006. Carroll *et al.*¹⁵

The possibility that field workers might develop antibiotic hypersensitivity from repeated massive exposure to antibiotic solutions used in spraying is indeed a likely occurrence. The two views in Figure 7-1 demonstrate clearly the exposure that sprayers undergo, both by direct exposure of the skin and by inhalation of antibiotic-containing field sprays. Frequent skin tests of field workers who have used streptomycin-containing sprays frequently over a four-year span suggest that streptomycin hypersensitivity may be developing in this group of individuals.¹⁶ However current data are inclusive and studies are being continued.



FIGURE 7-1. ILLUSTRATED ARE TWO TECHNIQUES OF APPLYING STREPTOMYCIN SPRAYS. Logue, J. T., Goldberg, H. S., Goodman, R. N., *The Public Health Significance of Antibiotic Residues in Foods*, Antibiotics Annual 1957-1958.

3. PUBLIC HEALTH HAZARDS FROM USE OF ANTIBIOTICS IN CONTROL OF ANIMAL DISEASES

Antibiotic preparations have proved to be as effective when used to eradicate animal infectious diseases as they have in the control of human infectious diseases. From the standpoint of public health, there seems to be little to fear from the adverse side effects of such treatment, since only well animals reach the consumer, and by that time there would be little chance of antibiotic residues remaining in the tissues from previous therapeutic administration.

Penicillin has become the most widely used antibiotic in the treatment of bovine mastitis, although streptomycin, neomycin, bacitracin and the tetracyclines have also been used. Detectable levels of penicillin are found in milk when milk from treated cows is marketed within a period

TABLE 7-9
PREPARATIONS ASSOCIATED WITH ANAPHYLACTOID SHOCK

	Cases	Deaths	Year anaphylactoid reactions occurred				
			1953	1954	1955	1956	1957
Unknown							
Penicillin Preparations							
Intramuscular injection*	611	63	24	179	231	301	14
Intramuscular with streptomycin or dihydro-streptomycin**	122	9					
Intravenous injection with streptomycin	1	0					
Tablets	35	0					
Troches	4	0					
Capsules	7	0					
Oral suspension	3	0					
Inhalation	4	0					
Ointment	1	0					
Nose drops	1	0					
Skin test	1	0					
Dental paste (with bacitracin, dihydrostrepto-mycin, and streptomycin)	2	0					
Syringe contaminated with penicillin	1	0					
Total penicillin preparation	793	72					
Other Preparations							
Streptomycin intramuscular	9	1					
Dihydrostreptomycin intramuscular	3	1					
Chloramphenicol capsules	1	0					
Tetracycline capsules	1	0					
Tetracycline in oil (oral)	1	0					
Tetracycline intramuscular or testosterone in-tramuscular	1	0					
Total other preparations	16	2					
Total all preparations	809	74					
		(9.1% deaths)					

of time less than seventy-two (72) hours after the last treatment. Pasteurization does not destroy or inactivate all of the penicillin present. Surveys of milk specimens have shown that in 1956, of 1,706 market specimens of milk, 5.9% contained penicillin in levels up to 0.5 units per millimeter. This represents a concentration of 550 I.U. per liter.¹⁷

A panel of some thirty specialists agreed that these residual penicillin levels should not be considered a hazard to the health of consumers, either by the likelihood of developing resistant pathogens through modification of intestinal flora, or by sensitizing non-sensitive people. The opinion was expressed, however, that the penicillin that exists in milk might well evoke allergic reactions in extremely sensitive individuals.¹⁸

Severe and often fatal anaphylaxis from penicillin, streptomycin and chlortetracycline administration is now a well recognized hazard in antibiotic therapy¹⁹ (see Table 7-9).

F. S. Thatcher²⁰ points to the rising incidence of chronic bovine mastitis due to penicillin-resistant staphylococci. In addition, he pointed out that, in 1953, milk and cheese-borne staphylococci were predominantly of phage group IV, rarely the cause of infection in man. More recent studies show an increasing occurrence of phage group III, phage type 81, penicillin-resistant staphylococci which commonly cause staphylococcal infections in humans.

In our eagerness to cure the lactating cow and increase our milk supplies, are we creating another avenue whereby our ever-strengthening enemy, the penicillin-resistant staphylococcus, can gain access to the population? In pasteurized products this danger probably does not exist; however, many cheeses contain viable staphylococci when consumed. This problem demands close scrutiny.

4. ANTIBIOTICS AS DIETARY SUPPLEMENTS

Antibiotics, namely chlorotetracycline and oxytetracycline, are now almost universally used as growth-promoting adjuncts in feeds for cattle, swine, sheep, and poultry. These antibiotics, when mixed in food rations at proper levels, do not have any harmful effects on the animals and usually result in increased growth.^{21,22}

Broquist and Kohler²³ point out that, following present commercial practices of nutritional feeding of chlortetracycline to farm animals in amounts of 10 to 20 g per ton of feed, the antibiotic was not present in the serum or tissues of the animals. When the antibiotic was fed to chickens or pigs for an extended time at levels above 50 g per ton, the antibiotic could be detected in the serum. At levels of 1,000 g per ton,

the antibiotic could be recovered from tissue in the order of 1 part per 10,000 parts of tissue. These trace amounts of chlorotetracycline resulting from massive chlorotetracycline dosage rapidly disappeared from the tissues when the antibiotic was removed from the diet one or two days prior to slaughter, or when the meat was cooked.

The exact mechanisms whereby antibiotics exert their growth-promoting effects are, as yet, poorly understood and are subject to considerable speculation. Antibiotics, when used as food supplements, do cause a marked alteration in gut flora with resultant proliferation of resistant organisms, mainly micrococci, proteus, pseudomonas, and yeasts. This is the same situation that is commonly observed in humans who receive therapeutic doses of these same antibiotics in treatment of human disease. Exactly how alterations of gut flora with proliferation of resistant organisms result in increased growth in growing animals is not known. The fact remains that antibiotic-treated feeds do increase meat production, and perhaps small quantities of antibiotic residues may be traced within present assay limits into the tissues, and are thus present for human consumption. The amount of antibiotic residues recovered from animal tissue is proportional to the amounts of antibiotic supplement in the food. Are these antibiotic residues of sufficient magnitude to cause trouble when consumed by humans? The answer to this question is of course unknown at this time.

Thus far, long-term low-level feeding (10–20 mg/day for 6 mos.-1 yr.) of antibiotic residues has not been attempted in human beings, although plans for such studies are under way. Long-term low-level feeding experiments in animals lead one to suspect that no untoward effects may be expected in humans. Thus far, antibiotics used as feed supplements have not led to untoward effects in man. Residues of bacteriologically-active antibiotics are not encountered in the flesh of animals that have received commercially recommended levels of antibiotics (up to 20 ppm) in feeds throughout their life span. Furthermore, detectable levels of bacteriologically-active antibiotic residues in the flesh of animals fed antibiotic supplements disappear rapidly when antibiotic feeding is discontinued for a few days prior to slaughter. There then would seem to be little public health danger from the use of antibiotics-supplemented feed, if feeding were discontinued after the maximum growth stimulation occurred and well before the time of slaughter. Jukes²⁴ has summed up the public health hazards of antibiotics used in animal nutrition very clearly as follows:

“The prolonged feeding of comparatively high levels of the common antibiotics to animals has not raised problems in public health as regards the consumption of animal products. Broquist and Kohler²³ found no detectable

amounts of chlortetracycline in the liver and muscle of chicks receiving chlortetracycline, 200 mg/kg of diet. Barely detectable amounts were present in the blood serum. At unusually high dietary levels, traces of the antibiotic were found in the liver and muscle. The values ranged from less than 0.05 to 0.165 mg/g of muscle when the diet contained 2 g/kg of chlortetracycline per kg of diet. Similarly with pigs, the level in the muscle tissue was 0.30 mg/g with 2 g of chlortetracycline per kg of diet and undetectable in the muscle with 200 mg/kg of diet. In further studies, Kohler and co-workers⁴ found that chlortetracycline in poultry meat was readily destroyed by cooking, and the destruction of chlortetracycline proceeded more rapidly than that of oxytetracycline. Chicken breast muscle containing 3 mg/g of chlortetracycline was found to be free from the antibiotic after boiling for fifteen minutes or after roasting for thirty minutes at 230°C. No toxic reactions are imaginable from the traces of chlortetracycline which might be present in the meat of animals fed high levels of the antibiotic. Indeed, these traces of antibiotic were only of the order of 50 mg/lb or less of muscle tissue even when the chicken received 200 g of chlortetracycline per ton of diet and the antibiotic was destroyed by ordinary cooking procedures."

B. UNTOWARD REACTIONS IN HUMANS FOLLOWING THERAPEUTIC USE OF ANTIBIOTICS

From the foregoing discussion, it is apparent that detectable levels of microbiologically-active residues of all of the antibiotics commonly used in food production, processing, and preservation may reach the unsuspecting consumer by a variety of ways. Thus far, we have inferred vaguely that antibiotics may be dangerous to the human on exposure or consumption, and at the same time we hail the antibiotics for enabling man to eradicate or control many of the infections that have long been a scourge to his well-being. What then are the untoward reactions that may appear when the unsuspecting food consumer comes in contact with food containing detectable levels of antibiotic residues?

To date, there is no evidence that low antibiotic residues, such as measured in the food products previously discussed, act the same in the human host as the antibiotic doses used in the treatment of human disease. With the possible exception that even minute amounts of an antibiotic can cause severe hypersensitivity reactions in a sensitized host, there is little evidence in the literature that low antibiotic residue levels have adverse effects on humans.

One must keep clearly in mind the fact that therapeutic levels are many times greater than those levels of antibiotic residues detected in foods. Then, too, the public health hazard that is to arise from the non-medical uses of antibiotics must occur from direct contact with the antibiotic residue or from ingestion of foods containing the antibiotic residue. We need not concern ourselves with the many untoward

reactions that have resulted from intramuscular or intravenous use of these agents in the treatment of disease. The medical literature is filled with reports of adverse reactions in humans from therapeutic doses of the antibiotics with which we are concerned, namely, penicillin, streptomycin, and the tetracyclines.²⁵

One must then confine one's interest to the specific antibiotic, the reactions produced by this antibiotic on direct exposure or by ingestion, and finally one must determine what dosage or exposure level is necessary to produce an untoward reaction in the human host.

1. PENICILLIN

Untoward reactions to penicillin have occurred from direct contact, from intramuscular and intravenous administration, and following oral administration. Reactions are extremely varied, ranging from severe local reactions in the skin, muscle, and mucous membrane to skin allergies such as urticaria, angioneurotic edema, erythema and eczema, and finally to generalized systemic reactions such as serum sickness, anaphylactic shock, and cardiovascular and renal disturbances. Allergic symptoms can occur explosively and very rapidly after exposure of the sensitized host to even minute amounts of penicillin. These symptoms can also occur from seven to fourteen days after exposure, constituting the so-called delayed allergic reaction, which is now well recognized by all who use these agents in the treatment of human disease.

Topical applications of penicillin are especially likely to cause sensitization in the human host. Such cases were reported for penicillin by Kolodny and Denhoff,²⁶ Hopkins and Lawrence²⁷ and Feinberg and Feinberg.²⁸ According to a survey made by the British Ministry of Health in 1953, 256 cases of topical sensitization to penicillin and streptomycin were discovered among those handling these drugs in 70 local health agencies and 76 chest hospitals and sanatoria. Of these cases, 80 were cases of skin sensitivity due to penicillin and 186 due to streptomycin.¹ Allergic reactions following skin contact with penicillin or its topical application are so numerous that the latter method of penicillin administration should be abandoned.

Closely related to the many manifestations of skin hypersensitivity produced by direct contact with penicillin are the systemic reactions such as serum sickness, anaphylactic shock and cardiovascular and renal complications.²⁵ Most of these more serious complications of penicillin therapy have been due to intravenous or intramuscular administration of high dosage levels of this drug; however, well documented cases of anaphylactic shock have been reported from the oral use of penicillin,^{29,30}

and during the local administration of penicillin-containing compounds.³¹ Bierlein³² observed the occurrence of an anaphylactic shock reaction after an intradermal test quantity of 0.000003 units of penicillin. A passive transfer test for penicillin in this patient was positive with 0.003 units of penicillin. Cases of anaphylactic shock and other reactions sufficiently severe to cause death do occur.^{33,34}

Since hypersensitivity reactions can be produced in the sensitized host by even minute amounts of penicillin, it is easy to recognize that milk containing levels of 0.5 units of penicillin per ml could certainly precipitate any of the above described reactions following ingestion by a previously sensitized person. To our knowledge no such cases have yet been reported.

2. STREPTOMYCIN

There is hardly a side effect accompanying the use of antibiotics which has not been observed following exposure to streptomycin. Allergic reactions such as urticaria, angioneurotic edema, eczema, exfoliative dermatitis, serum sickness and anaphylaxis have been described.^{35,36,37}

Contact dermatitis due to streptomycin hypersensitivity has been reported repeatedly following exposure of sensitized individuals to minimal amounts of streptomycin. This has been a particularly distressing problem to nursing personnel.³⁸

In addition, neurotoxic eighth cranial nerve injuries are quite common following prolonged therapeutic use of streptomycin.³⁹ Severe kidney damage with uremia and lower nephron nephrosis have also been recognized as toxic side effects following administration of streptomycin.²⁵

3. CHLORTETRACYCLINE-OXYTETRACYCLINE-TETRACYCLINE

Skin hypersensitivity reactions⁴⁰ such as urticaria, angioneurotic edema,⁴¹ erythema and itching eczema have been following use of the tetracyclines.^{42,43} These reactions most commonly follow oral administration of the tetracycline drugs, although these reactions can follow topical, intramuscular, or intravenous administration of the tetracyclines. Serum sickness following oral administration of the oxytetracycline,⁴⁴ and angioneurotic edema following oral administration of chlortetracycline⁴⁵ have been reported. Oral, pharyngeal, vulvovaginal and anal mucous membrane lesions are quite common after exposure to this group of drugs. Mucous membrane hemorrhage, black hairy tongue, overgrowth of yeast, fungi and changes suggestive of pellagra have been described following local application of oxytetracycline and following the use of chlortetracycline tablets or lozenges.^{46,47} Turrell and Maynard reported

125 cases of so-called "anorectocolonic syndrome," i.e. diarrhea and perianal pruritus which resulted from oral treatment with one of the broad spectrum antibiotics.⁴⁸

C. SUPERINFECTION FOLLOWING ANTIBIOTIC THERAPY

1. STAPHYLOCOCCUS

Severe gastro-enterocolitis due to antibiotic-resistant *Micrococcus pyogenes* var. *aureus* (staphylococcal gastro-enterocolitis) has resulted from treatment with penicillin, streptomycin, chlortetracycline, oxytetracycline and tetracycline. This unwelcome, serious, and often fatal complication of antibiotic therapy has been recognized with increasing frequency following therapy with the broad spectrum antibiotics. It may occur during medical treatment for an infection, during preoperative preparation of a patient for abdominal surgery or during the postoperative period. It may result from oral or parenteral administration of the drug. Oral doses of the broad spectrum antibiotics that have caused this complication are usually in excess of 1.5 to 2 grams daily in adults, and usually do not occur with less than three days of continuous antibiotic treatment.^{48,49,50} A fatal case following a total oral dose of 2 g of tetracycline hydrochloride has been reported by Marcorelles.⁵¹

2. MONILIASIS

Prolonged therapy with broad spectrum antibiotics, penicillin and streptomycin, often results in suppression of the normal bacterial growth with a resulting overgrowth of resistant yeasts and fungi.^{52,53} Overgrowth of fungus may be localized in the pharynx, gastrointestinal tract⁵⁴ or lungs⁵⁵ or may appear as widespread fungus septicemia.⁵⁶

In an interesting study on the appearance of monilia in the sputum, feces and rectum during treatment with oxytetracycline, Sharp⁵⁷ showed that successive cultures on patients revealed an increasing number of positive cultures for *Candida albicans* over a five day course of oxytetracycline with a mean total dose of 14.2 grams per patient.

3. PROTEUS-PSEUDOMONAS

In some patients receiving broad spectrum antibiotics, usually those antibiotics which suppress the growth of gram-negative enteric bacteria, super infections with *Proteus* and *Pseudomonas* organisms can occur. These organisms are usually antibiotic resistant non-pathogenic in-

habitants of the normal human skin and mucous membranes of the respiratory and gastrointestinal tracts, and apparently occasionally become pathogenic when the normal balance of bacterial flora is upset by antibiotic therapy. Underlying structural abnormalities such as urinary tract strictures, prostatic obstructions, pyelonephritis, and bronchiectasis, tumors and other infectious processes seem to predispose the human body to infections with these organisms.⁵⁸

It is unlikely that the antibiotic residue levels as measured in foods ready for consumption could suppress normal enteric bacterial growth sufficiently to result in superinfections with staphylococci, candida, *Proteus* or *Pseudomonas* in an otherwise healthy, but unsuspecting food consumer even if residue levels were ingested daily for some time. Well controlled studies on human volunteers must be carried out before this problem can be resolved.

D. ANTIBIOTIC RESIDUES AND MICROBIAL RESISTANCE

The advent and subsequent use of large doses of antibiotics in the control of human infectious disease has brought sharply into focus certain disadvantages. One of the most important and increasingly serious of them is the recognition that certain bacteria, originally sensitive to an antibiotic, can develop resistant strains on continued contact with the antibiotic.^{59,60} Eagle has demonstrated that the resistance of sensitive bacteria can be increased by increasing the concentration of antibiotic to which the bacteria is exposed. With human pathogenic bacteria, the development of resistance has been demonstrated *in vitro* to concentrations of penicillin, streptomycin, chlortetracycline and oxytetracycline in levels as low as the antibiotic residue levels measured in foods.⁶¹ The development of penicillin resistance by the staphylococcus, and the ability of the staphylococcus to develop resistant strains to each new effective antibiotic produced, has been a continuing puzzle to all who work with these organisms.⁶² When penicillin was first introduced into human therapy, almost all of the strains of staphylococcus were sensitive to this drug. At the present time more than seventy-five per cent (75%) of the strains isolated from human infections are resistant to penicillin.⁶³ Perhaps more alarming has been the recognition that penicillin-resistant organisms are being carried by increasing numbers of normal people both in hospitals and the community at large.^{64,65,66}

Streptomycin and dihydrostreptomycin have long been the mainstay in the effective antibiotic treatment of human tuberculosis. Here again the rapid appearance of streptomycin resistance by the *Myco-*

bacterium cause health authorities and physicians to redouble their efforts to prevent the appearance and further spread of resistant tubercle bacilli. Streptomycin resistance in mycobacterium is permanent, once it occurs. This indeed creates a potentially disastrous public health hazard.

In similar fashion many sensitive bacteria, particularly staphylococci, may develop resistance on continued exposure to chlortetracycline and oxytetracycline.

The development of microbial resistance to therapeutic doses of penicillin, streptomycin, chlortetracycline and oxytetracycline is well documented. An excellent review of the literature on this problem is presented by Finland.⁶⁷

Penicillin given parenterally and orally does not reduce the number of bacteria in the feces, and the total count of aerobes and coliforms may increase.⁶⁸ Continuous oral doses of penicillin cause a decrease in gram-positive organisms, particularly streptococci in the fecal flora; however, no change in the range of sensitivity was found when compared with organisms encountered before penicillin treatment.⁶⁹

Escherichia coli is suppressed or eliminated during the first two days of oral therapy with streptomycin, unless streptomycin-resistant strains were present, or unless they appeared rapidly.⁷⁰ When strains of *Escherichia coli* reappear they are usually highly streptomycin-resistant.⁷¹

Chlortetracycline and oxytetracycline cause a rapid reduction of coliforms during treatment,⁷² but significant resistance apparently does not result.⁷³

The pertinent question at hand is whether antibiotic residues as measured in foods cause similar changes in human flora after prolonged exposure of the flora to the residue levels in food. The answer to this question is unknown at the present time. Data from experimental animals suggest that long-term low-level feeding of streptomycin at levels of 20 mg daily can cause emergence of significant streptomycin resistance in the normal intestinal flora of white Swiss mice. Guinea pigs fed streptomycin up to 35 mg daily for a three month period showed no increased resistance in oral flora over that of control pigs.⁷⁴ Similar feeding studies have shown that chlortetracycline does not cause detectable emergence of resistance by coliforms in mice. Some resistance does occur with lactose-negative enteric bacteria (*Proteus-Pseudomonas*); however, these levels of resistance are minimal, and the percentage of organisms exhibiting resistance is low when compared with the above-reported studies with streptomycin.⁷⁵

The appearance of penicillin-resistant staphylococci in milk from infected udders treated with penicillin has been commented on by

Thatcher.²⁰ These organisms probably developed resistance to the high therapeutic doses of penicillin used in treating bovine mastitis and not from the decreasing penicillin residues in subsequent samples of milk. This point should be clarified.

Carefully-controlled long-term feeding studies on the human gastrointestinal flora with penicillin, streptomycin, chlortetracycline, and oxytetracycline residues must be carried out before relaxing our rigid controls over these antibiotics in food preservation. The wisdom of prohibiting the further use of penicillin in the treatment of bovine mastitis would certainly seem apparent. Other antibiotics are effective in controlling this disease without running the risk of hypersensitivity reactions in the vast reservoir of penicillin-sensitive milk consumers.

E. SUMMARY AND CONCLUSION

The foregoing discussion and its supporting literature permit a number of current conclusions about the public health aspects of the non-medical uses of antibiotics. A number of pertinent unanswered questions also emerge.

The antibiotic residues of penicillin, streptomycin, chlortetracycline and oxytetracycline may find their way into the human diet in detectable levels through the previously discussed channels. Antibiotics used as food supplements in the raising of cattle, swine, and poultry may exist in the meat and eggs when eaten. Antibiotics used to control animal diseases have in some instances left detectable residues in the food product, and antibiotics used experimentally in food preservation can exist in detectable amounts in the foods when consumed. The large concentrations of antibiotics used in plant disease control and food processing expose many workers to the risks of topical sensitivity to the antibiotics, and the resulting risk of severe hypersensitivity reactions. In view of the extensive literature covering the various serious side effects of antibiotic therapy, hypersensitivity, toxicity, superinfection and the emergence of microbial resistance, one must conclude that it is the very low levels of antibiotic residues which are responsible for the lack of adverse side effects above mentioned. Emergence of antibiotic-resistant bacterial flora in areas where large concentrations of antibiotics have been used may yet complicate the problems one tries to solve by the use of these agents.

Previous experiences with antibiotics have shown that untoward effects of antibiotics usage depend on the specific antibiotic, the dosage, and the nature of the host. Therefore, attention must be paid to each antibiotic individually, and to the effects it produces in the human, either by ingestion or by topical exposure.

Penicillin may exist in milk in levels of 550 I.U. per liter. This level is sufficient to cause a severe sensitivity reaction in a highly sensitive host. Penicillin-resistant staphylococcal infections are becoming more prevalent in our dairy herds. In view of these two established facts, the wisdom of removing penicillin from mastitis preparations becomes apparent.

Experimentally, streptomycin exists in levels of 25 mcg/ml in fresh-cut vegetables treated to prevent bacterial decay. These levels are not destroyed by cooking. Hypersensitivity reactions and prompt emergence of streptomycin-resistant bacterial flora to the streptomycin residue levels are not unlikely. Cumulative toxicity from continued exposure to residue levels of streptomycin has not occurred, and clinical experience would lead one to believe that it would not occur from residue levels. This point demands continued study in human volunteers. Streptomycin used in plant disease control apparently does not result in detectable residues in the food products when marketed. Streptomycin should not be used in food preservation because of its relative heat stability and high sensitizing potential. Its use in plant disease control, on the other hand, would seem perfectly acceptable as long as the workers who use the streptomycin-containing preparations are aware of the hazards. Streptomycin would thus be considered, from a public health standpoint, in the same light as any other spray.

Chlortetracycline and oxytetracycline have thus far been the most useful agents used in food production, food processing, and food preservation. The residue levels of these antibiotics are very small, and the unstable nature of the residues in food products indicates that adverse side effects from continued exposure to these low residue levels will not result in adverse side effects in the food consumer.

Adequate scientific studies to support the above conclusions have not been carried out to date. Residues of penicillin, streptomycin, chlortetracycline and oxytetracycline should be fed to a variety of experimental hosts, and the emergence of antibiotic resistance in oral and enteric flora determined. Concurrently, standard toxicological studies should be carried out to determine whether low-level, long-term feeding of antibiotic residues interferes with normal physiological functions. Thus, tests of liver function, kidney function, blood studies and metabolic studies could be analyzed for cumulative toxicity resulting from constant antibiotic residue intake.

Similar long-term feeding studies must be carried out in human volunteers before one can decide whether or not a true public health hazard exists from exposure of the public to food supplies containing antibiotic residues. One must conclude that the seriousness of the

public health hazards of antibiotic residues in food has been overstated; however, a continued conservative attitude should be maintained until definite answers to the foregoing problems emerge.

REFERENCES

1. Ross, J. M., 1957, Antibiotics in relation to public health. *The Veterinary Record*, Vol. 69, No. 8, Issue No. 41117, 163-292.

This excellent review of the public health hazards from non-medical uses of antibiotics was presented in the proceedings of the conference on supplements and additives in animal feeding-stuffs organized by the British Veterinary Association of Victoria Halls, Bloomsburg Square, London, W.C.I. on November 22 and 23, 1956.

Dr. Ross discusses adequately the therapeutic hazards of antibiotic therapy, microbial resistance, sensitization, allergy and toxicity. Short discussions of antibiotics as dietary supplements and as food preservatives are included to point up his discussion on the relationship of antibiotics to public health. In the discussion that followed this excellent paper, Dr. McKay (London) made the following amusing but very intelligent observation: "It made one shudder to think of all the snacks one has had over the years of biscuits, cheese and beer because they must have contained considerable quantities of antibiotics."

2. Tarr, H. L. A., 1956, Control of bacterial spoilage of fish with antibiotics. *National Academy of Sciences Publication* 397, 199.

A review of the efforts at controlling bacterial spoilage in fish from 1938 to the present. Data presented shows that chlortetracycline hydrochloride added to storage ice or refrigerated sea water in concentrations of 1 mg/ml possess outstanding bacteriostatic properties. Antibiotic residue penetration of fish flesh thus stored was determined in low levels. These low residue levels were inactivated by heat. Evidence that chlortetracycline sensitive fish organisms may develop resistance when exposed to chlortetracycline in 1 mg/ml concentrations over a prolonged period of time is also presented. A brief discussion of chlortetracycline in preservation of whale flesh concludes this comprehensive paper.

3. Ziegler, F. and Stadelman, W. I. D., 1955, *Food Tech.* No. 1, 9: 107.

Poultry, dressed, washed, cooled and dipped for ten minutes in chlortetracycline solution, keeps longer without bacterial spoilage than control birds.

4. Kohler, A. R., Miller, W. H. and Broquist, H. P., 1955, *Food Tech.* No. 1, 9: 151.

Comparative studies of bacteriostatic properties of a number of antibiotics indicate that chlorotetracycline is most effective agent for use in poultry preservation. Resulting chlortetracycline residues in poultry flesh are shown to be inactivated by heat.

5. Gibson, F. S., Kohler, A. R. and Hines, L. R., 1955, Ags. 15th Annual Meeting Inst. Food Technologists No. 46, *Food Tech.* 9, No. 5.

Further evidence that chlortetracycline is the most effective antibiotic for use in poultry preservation.

6. Durbin, C. G., Dilorenzo, J. J., Randall, W. A. and Wilner, D., 1954, Antibiotic concentration and duration in animal tissues and fluids II chicken blood tissue and eggs. *Antibiotics Annual 1953-1954*. Medical Encyclopedia, Inc.

Ninety to one hundred per cent of chickens fed a diet containing 50 and 100 ppm chlortetracycline had a small amount of chlortetracycline in the blood and flesh. Eggs from hens fed a diet of 200 ppm chlortetracycline had detectable levels of the drug. Data on inactivation of chlortetracycline by heating flesh and eggs is presented.

7. Goldberg, H. S. Weiser, H. H. and Deatherage, F. E., 1953, Studies on meat. IV. The use of antibiotics in preservation of fresh beef, *Food Tech.* **7**, 165.

Controlled bacteriological studies of fresh ground beef treated with chlortetracycline in both refrigerated and unrefrigerated states indicate that chlortetracycline enhances the keeping qualities of fresh ground beef by suppressing bacterial growth.

8. Weiser, H. H., Goldberg, H. S., Cahill, V. R., Kunkle, L. E. and Deatherage, R. E., 1952, *Food Tech.* **12**, vol. VII, no 12, 495-499.

Controlled bacterial population studies of fresh beef rounds infused with chlortetracycline solution indicate that treated rounds kept longer without deep spoilage due to bacterial growth where refrigeration is delayed. Infusion of whole animals antemortem with chlortetracycline gave similar results. Data on the disappearance of chlortetracycline from beef so treated are presented.

9. Becker, R. F., Goodman, R. N. and Goldberg, H. S., Prolonging the shelf life of refrigerated prepackaged spinach with antibiotics. *Antibiotics Annual 1957-1958*, 224. Medical Encyclopedia, Inc.

Oxytetracycline, chlortetracycline and streptomycin sulfate in 25 mg/ml solution retarded decay development caused by *Erwinia carotovora* in fresh pre-packaged spinach. Cooking destroys residue levels of oxytetracycline and chlortetracycline but not streptomycin.

10. Goodman, R. M., Johnson, M. R. and Goldberg, H. S., Residual quantities of antibiotics detected in treated plant tissue. *Antibiotics Annual 1957-1958*, 236. Medical Encyclopedia, Inc.

Data on oxytetracycline residues in potatoes and cabbage and streptomycin residues in spinach are presented. Oxytetracycline residues are shown to be heat labile, while streptomycin residues persist despite exposure to live steam for three minutes.

11. Goodman, R. N. and Johnston, M. R., Stability of streptomycin in apple and potato tissue. *Antibiotics Annual 1956-1957*. Medical Encyclopedia, Inc.

Capsules containing 375 mg of streptomycin were inserted and sealed in holes in apple-tree trunks. Assay of mature fruit three weeks later revealed the presence of heat stable streptomycin residues. Potatoes are dipped for one minute in streptomycin solution (250 mg/ml) and stored. After 120 days of dry storage heat-stable streptomycin residues in the levels of 16.5 mg/ml were detected.

12. Brody, H. D. and Francis, F. J., 1956, The effect of streptomycin on pre-packaged spinach. *Proceedings of American Society of Horticultural*

Science, Annual Conference of AIBS Storrs, Connecticut, August 26-29 (abst).

Spinach dipped in streptomycin at a concentration of 500 mg/ml for control of soft rot (*Erwinia carotovora*) resulted in a residual level of 25 mg/ml. These residues did not decrease appreciably after cooking.

13. Murneek, A. E., 1952, Thiolutin as a possible inhibitor of fireblight. *Phytopath.* **42**, 57.

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- ✓ 14. Morgan, Billie S., Goldberg, H. S. and Goodman, R. N., Residual quantities of a streptomycin-oxytetracycline combination as determined by a simple microbiologic assay. *Antibiotics Annual 1954-1955*. Medical Encyclopedia, Inc.

A simple, rapid microbiologic assay for the combined residue of streptomycin-oxytetracycline is described. Persistence of residue levels on apple leaves after spraying is demonstrated. The absence of residue levels in mature fruit samples is also shown.

- ✓ 15. Carroll, V. J., Koch, G. and Kersey, R. C., Estimation of micro-quantities of streptomycin and oxytetracycline in tomatoes. *Antibiotics Annual 1955-1956*. Medical Encyclopedia, Inc.

Microbiologic assay to measure the separate residue levels of streptomycin and oxytetracycline is described. This assay technique shown to be sensitive for the detection of as little as 0.5 ppm streptomycin and as little as 0.025 ppm oxytetracycline in tomatoes. The absence of detectable residue levels in mature tomatoes from treated plants is shown.

16. Logue, J. T., Goldberg, H. S. and Goodman, R. N., The public health significance of antibiotic residues in foods. *Antibiotics Annual 1957-1958*, 333. Medical Encyclopedia, Inc.

Standard tuberculin-type intradermal skin testing using 500 units of streptomycin with suitable controls in opposite arms was carried out in thirty agricultural workers who had used streptomycin sprays frequently over a three- to four-year period. Two workers had a strongly positive skin test to streptomycin, manifested by erythema and a palpable wheal.

17. Welch, H., 1957, *Am. J. Public Health* **47**, 701.

The results of milk surveys for residual penicillin during the years 1954, 1955 and 1956 are presented. In the first survey (1954) ninety-four quarts of fluid milk were tested for antibiotic activity. Three quarts (3.2%) were found positive for penicillin. In the second survey (1955) 11.6% of 474 quarts of market milk were found positive for penicillin in concentrations from 0.003 units/ml to 0.08 unit/ml (highest amount 80 units/liter). In a third survey (1956) (1,706 samples from all 48 states and the District of Columbia), 5.9% were positive for penicillin in concentration of 0.003 to 0.550 units/ml (highest 550 units/liter).

The above studies on penicillin residues in milk were discussed.

18. Welch, H., 1957, *Food Drug Cosmetic Law J.* **12**: 462.

Dr, Welch, the director of the division of antibiotics, United States Food and Drug Administration, discusses the effects of antibiotics

on food for human consumption and the effects of antibiotics used in animal feeds and drugs. Dr. Welch points out that antibiotic residues must be shown to be non-detrimental to the health of the general public prior to antibiotic use as food preservatives. This requirement is contained in the Miller Amendment which specifically controls antibiotic usage in food supplies in the United States.

19. Welch, H., Lewis, C. N., Weinstein, H. I. and Breckman, B. B., Severe reactions to antibiotics. *Antibiotics Annual 1957-1958*, 296. Medical Encyclopedia, Inc.

A comprehensive survey of 800 hospitals and 1,600 physicians to analyze cases of severe reactions to antibiotics. A total of 3,419 case histories were reviewed all of which were examples of severe reactions to antibiotics.

20. Thatcher, F. S., 1958, *Canadian Journal of Public Health* 49, No. 2.

A timely discussion of the public health aspects of antibiotic uses in food preservation, the mode of action of antibiotics and the problem of antibiotic resistance. This author points up the serious problems brought about by the treatment of bovine mastitis with penicillin, and the increasing appearance of penicillin-resistant staphylococci in milk and cheese from such treatment.

21. McGinnis, James, 1956, Response of poultry to antibiotic supplementation of feeds. National Academy of Sciences Publication 397, 3.

An excellent review of the literature on the growth-promoting effects of antibiotics in poultry. The evidence discussed suggests that the growth-promoting effect results from the suppression of sensitive bacteria which may be causing sub-clinical or unrecognized disease in poultry flocks.

22. Cunha, T. J., 1956, Antibiotics for swine, beef cattle, sheep and dairy cattle. National Academy of Sciences Publication 397, 9.

An excellent review of the literature on the growth-promoting effects of antibiotics in swine, beef cattle, sheep and dairy cattle. The author concludes from the data discussed that the effective antibiotics mixed in the ratio at proper levels do not have any harmful effects, and are usually beneficial to beef cattle and sheep.

23. Broquist, H. P. and Kohler, A. R., Studies of the antibiotic potency in the meat of animals feed chlortetracycline. *Antibiotic Annual, 1953-1954*, 409. Medical Encyclopedia, Inc.

Authors Summary:

"Eight-week-old chickens were fed 20 to 6,000 g chlortetracycline (CTC) per ton of feed for one week, and the CTC content of the serum was determined by microbiological assay. Detectable amounts (0.02 mcg/CTC/ml or greater) of antibiotic were found in the serum when 600 g CTC or greater were fed per ton.

Baby chicks received feed containing 50 and 100 g CTC per ton for 11 weeks. The serum contained 0.014 mcg to 0.061 mcg CTC per ml, but no antibiotic was detected when the antibiotic was withdrawn from the diet for one day. Similar results were obtained with pigs.

Eight-week-old chickens were fed a diet containing 200 g per ton of CTC for one week. The breast muscle contained 0.02 mcg CTC per g.

but none was present when the meat was cooked or when the antibiotic was withdrawn from the diet for two days.

When CTC was added to chicken muscle to at least 1,000 times the level obtained by feeding, the antibiotic was still destroyed by cooking.

Hens on diets containing 20 to 200 g per ton of CTC laid eggs in which no CTC was detected, nor could a rickettsiostatic effect be demonstrated when murine typhus was cultivated in these eggs. When 2,000 g per ton CTC were fed to hens, such eggs contained low but variable amounts of CTC and a rickettsiostatic effect was obtained."

24. Jukes, Thomas H., Antibiotics in nutrition. Antibiotics Monographs/4.

An excellent, comprehensive monograph covering the field of antibiotics in animal nutrition. Chapters on the mechanism of the antibiotic growth effect, the effect of feeding antibiotics on the requirements for nutrients, antibiotics in animal production and the physiologic and other effects of dietary antibiotics make this monograph outstanding.

25. Schindel, Leo, 1957, Unexpected reactions to modern therapeutics. Charles C. Thomas, Springfield, Illinois.

Authors flyleaf comment:

"The first part of this book deals with unexpected reactions to antibiotics, and describes signs and symptoms due to these reactions. Attention is called to the "big" antibiotics like penicillin, streptomycin, chloramphenicol; the broad spectrum antibiotics such as chlortetracycline, oxytetracycline and tetracycline itself; and finally, erythromycin, neomycin, bacitracin, fumagillin, novobiocine, cycloserine and polymyxin are discussed.

One chapter is devoted exclusively to diseases due to antibiotic-resistant bacteria, an entirely new entity since the advent of antibiotics, which has provided the medical profession with a host of clinical and therapeutic problems hitherto unknown. Emphasis is put on the management of unexpected reactions.

This survey represents material gathered from many hundreds of publications from all parts of the world, and it is hoped that as more becomes known about these reactions they will be more easily prevented. The general practitioner as well as the specialist will find it of immense interest and value."

26. Kolodny, M. H. and Denhoff, E., 1946, Reaction to penicillin therapy. *J.a.m.a.* 130; 1958.

A well-studied group of 124 skin reactions to sodium penicillin. Sixteen per cent (16%) of this group showed immediate reactions. Patients with skin diseases showed a much higher incidence of reaction than non-dermatologic patients.

27. Hopkins, J. G. and Lawrence, H., 1947, Sensitization to penicillin. *Journal of Allergy* 18; 251.

Fifty-six (56) out of four hundred ninety-two (492) patients who received penicillin locally reacted with contact dermatitis.

28. Feinberg, S. A. and Feinberg, A. R., 1956, Allergy to penicillin. *J.a.m.a.* 160; 778.

An excellent discussion of penicillin allergy, its many clinical manifestations, the immunologic mechanisms involved, and the overall incidence. The point is made that a person who has once had a peni-

cillin reaction is more likely to experience a similar reaction a second time, but nevertheless a large percentage of such people can take second injections with impunity. The sensitive dose of penicillin may be given months after initial penicillin exposure. The allergenic quality of penicillin is not destroyed by heat. The amount of penicillin in cows milk subsequent to treatment for bovine mastitis may constitute a sensitizing source of penicillin. A brief discussion of skin testing for penicillin hypersensitivity, and of the therapy of these reactions is also included.

29. Pierce, A. F., 1953, An anaphylactic reaction due to oral penicillin. *J. nat. m.a.* **45**; 68.

A case report of a 27-year-old white male who developed shock minutes after oral ingestion of 600,000 units of penicillin.

30. Welch, H., Lewis, C. N., Kerlan, I. and Putnam, L. E., 1953, Acute anaphylactoid reactions attributed to penicillin. *Antibiotics and Chemotherapy* **3**: 891.

These authors report the results of a survey in which a total of 63 anaphylactic reactions were reported, none of which had been reported previously.

1. Injection of procaine penicillin	55	(18 fatal)
2. Injection of penethamate (Neo-penil)	1	(recovered)
3. Injection of penicillin O	2	(recovered)
4. Oral tablet (dibenzylethylendiamine) dipenicillin G	1	(fatal)
5. Intrathecal injection of streptomycin	3	(1 fatal)
6. Injection of streptomycin	1	(recovered)
	<hr/> 63	

31. Blanton, W. B. and Blanton, F. M., 1953, Unusual penicillin hypersensitivity. *J. Allergy* **24**: 405.

An interesting case report of a 35-year-old drug detail man with known hypersensitivity to penicillin, who developed severe dyspnea, coughing, wheezing and rhinorrhea when penicillin G was administered by aerosol to another patient behind a closed door in another room. Severe skin reactions to patch tests of penicillin O in concentrations of 10 units, 100 units and 1,000 units were present in this patient. These patch tests caused a sense of constriction in the chest, sneezing and coughing in this patient.

32. Bierlein, K. J., 1956, Repeated anaphylactic reaction in a patient highly sensitive to penicillin. *Ann. Allergy* **14**: 35.

A case report of a 55-year-old nurse with multiple drug hypersensitivity reaction is described. The occurrence of anaphylaxis after intradermal injection of 0.000003 units of penicillin seems well established in this case.

33. Waldbloht, G. L., 1949, *Journal American Medical Association* **139**: 526.

A case report of a 39-year-old white female with a long allergic history and a previous history of intramuscular penicillin therapy, who collapsed and died immediately after injection of 50,000 units of penicillin.

34. Robinovitch, J. and Snitkoff, M. G., 1948, *Journal American Medical Association* **138**: 496.

A case of a 72-year-old white female who developed acute exfoliative dermatitis seven days after cessation of penicillin therapy for perforated appendicitis.

35. Cohen, A. C. and Glinsky, G. C., 1949, Cutaneous lesions occurring in the course of streptomycin therapy. *Arch. Derm. and Syph.* **60**: 373.

These authors describe the cutaneous eruptions occurring during streptomycin therapy in 137 patients in a VA Hospital during a 2 year period. Exfoliative dermatitis was the only eruption which demanded the cessation of therapy. Contact dermatitis was not an uncommon occurrence in nursing personnel of this hospital.

36. Feldman, M. D., 1948, Contact dermatitis from topical application of streptomycin ointment. *Arch. Derm. and Syph.* **57**: 1048.

Authors Summary:

"A case of contact dermatitis of the lower extremities is described. The reactions to patch tests with streptomycin were positive."

37. Hovding and Utna, 1956, Hypersensitivity to streptomycin. *Nordisk Medicine*, Foreign letter Norway, *J.a.m.a.* **161**: 263.

These authors report an incidence of streptomycin hypersensitivity in 29 out of 257 nurses who, in the course of their work, had been in contact with streptomycin, dihydrostreptomycin or both. Nineteen out of twenty nine had positive skin tests to a 20% solution of streptomycin or dihydrostreptomycin.

38. Crofton, J. and Foreman, A. M., 1948, Streptomycin dermatitis in nurses. *British Medical Journal* **2**: 71.

Authors Summary:

"Four nurses out of approximately 80 handling streptomycin at Brompton Hospital, London, during a period of 18 months, developed a dermatitis. Their exposure varied from six weeks to six months.

The dermatitis consisted of an itchy papular erythema of the skin, round the eyes and over the elbow flexures. In only one nurse were the hands involved.

Skin sensitivity tests, using 50 mg of streptomycin in 0.1 ml of saline, were positive in the four affected nurses but negative in controls.

People handling streptomycin should wear rubber gloves and avoid soiling their skin with the materials."

39. Chase, J. S., 1941, Toxic effects of streptomycin on the eighth nerve. *Am. Rev. Tuberc.* **56**: 418.

In patients undergoing streptomycin therapy, audiograms showed that in the majority of cases studied no appreciable hearing loss was present.

Vestibular function is definitely affected. Fifteen (15) out of 18 patients receiving 2 g or more of streptomycin daily for 90 days lost vestibular response. The loss of vestibular response is manifested by vertigo on moving the head, ataxia and dizziness.

40. Peck, S. M. and Feldman, F. F., 1950, Sensitivity reaction to aureomycin. *J.a.m.a.* **142**: 1137.

Three cases of patients with allergic eruptions due to aureomycin are reported. One eruption was urticarial in type. The second case

presented erythema-multiforme-like eruptions and urticaria, and the third case presented an eczematoid eruption of the groin and scrotum.

41. Parets, S. M., 1950, Angioneurotic edema and rash due to aureomycin. *J.A.M.A.* **143**: 653.

Authors Summary:

"A patient with multiple sensitivities was treated with 2 g of aureomycin hydrochloride daily for early bronchiectasis. A severe reaction consisting of angioneurotic edema of the face and a fairly generalized non-pruritic erythematous skin eruption, appeared on the seventh day of treatment and lasted one week. Treatment with various anti-histaminic drugs was of no benefit.

The literature dealing with the toxic effects of orally administered aureomycin has been reviewed briefly and some less known but significant side actions for which one should watch are described. Sensitization to aureomycin apparently can occur."

42. Harris, D. D., 1950, Aureomycin and chloramphenicol in brucellosis with specific reference to side effects. *J.A.M.A.* **142**: 161.

One hundred thirty-five (135) patients with chronic brucellosis were treated with chlortetracycline and chloramphenicol to a total dose of 25 g in ten days. Increased appetite, nausea, vomiting, epigastric distress, heart burn, and diarrhea were noted in 60% of the patients. Mucous-membrane ulceration, vesiculo-papular eruption and changes suggesting riboflavin deficiency were also noted. Overgrowth of intestinal *Candida albicans* occurred.

43. Putnam, C. E., Hendricks, F. D. and Welch, H., 1953-54, Tetracycline, a new antibiotic. *Antibiotics Annual*, 88. Medical Encyclopedia, Inc.

These authors discuss the properties of tetracycline. Blood concentrations following various oral doses were compared with those obtained using similar doses of chlortetracycline and were found to be comparable. Following multiple doses, tetracycline does accumulate in the blood.

Four of 217 subjects exhibited what may have been allergic response to the drug. Nausea, vomiting, and diarrhea occurred in 5.5% of 217 healthy male subjects administered 2 g of tetracycline daily for three days.

44. Johnston, T. G. and Cazort, A. G., 1953, Severe serum sickness reaction with cyanosis following terramycin. *Antibiotics* **3**: 484.

A case report of serum sickness following oral administration of terramycin.

45. Gittell, G., 1951, Unusual reaction to aureomycin. *J. A. M. A.* **147**: 1141.

A case report of a 36-year-old female who developed angioneurotic edema, urticaria and a shock-like state with hypotension, collapse, weakness and syncope, following a total oral dose of 3.75 g aureomycin in three days.

46. Kutscher, A. H., Budowsky, J., Lane, S. L. and Chilton, N. W., 1952. Reaction following the use of terramycin troches. *Journal of Allergy* **23**: 177.

A report on the incidence and types of reactions encountered following the use of terramycin troches. Reactions were noted in 76 (53%) of 143 patients treated.

47. Kutscher, A. H., Budowsky, J., Lane, S. L. and Chilton, N. W., 1953, Reactions following the use of aureomycin and procaine-penicillin troches. *Journal Allergy* **24**: 164.

A report on the incidence and types of reactions encountered in 300 patients during and after medication with crystalline aureomycin hydrochloride troches, or crystalline procaine penicillin G troches, or placebo troches.

51% of patients had some type of reaction to aureomycin

38% of patients had some type of reaction to penicillin

In control studies 7% of the patients had complaints, but no physical evidence of reactions.

48. Turell, Robert and Maynard, Aubre de L., 1954, Anorectocolonic side effects of antibiotic therapy. *J. A. M. A.* **156**: 217.

These authors describe 136 cases of "anorectocolonic syndrome" (diarrhea, perianal pruritis, ano-genital soreness and burning). In 125 cases these symptoms occurred following chlortetracycline or oxytetracycline therapy, 250 mg every 6 hours from three to eight days. In eleven patients these symptoms occurred following erythromycin administration. Representative case reports and a discussion on treatment are included.

49. Fowler, B. J., 1955, Postoperative staphylococcal enterocolitis during antibiotic therapy. *British Medical Journal* **1**: 1313.

A report of three well-studied cases of fatal postoperative staphylococcal enteritis which occurred during treatment with penicillin and streptomycin. The classical clinical picture of sudden severe diarrhea, collapse, vomiting, and fainting associated with recovery of resistant staphylococci is discussed.

50. Austrian, Robert, 1955, Staphylococcal enteritis as a complication of antibiotic therapy. *New York State Journal of Medicine* **55**: 2475.

An excellent general discussion of the problem of drug-induced staphylococcal enteritis, its clinical features, treatment and prevention. The use of erythromycin, bacitracin, neomycin and chloramphenicol is discussed at some length. More effective control of persons carrying antibiotic-resistant staphylococci is urged.

51. Marcorelles, J., 1955, Fatal acute enterocolitis after absorption of tetracycline. *Archives des maladies de l'appareil digestif et des maladies de la nutrition* **44**: 1086 (Sept.-Oct.)

A well-documented case of fatal enterocolitis following absorption of a total dose of 2 g of tetracycline hydrochloride.

52. Pappenfort, R. B. and Schnall, E. S., 1951, Moniliasis in patients treated with aureomycin. *Arch. Int. Med.* **88**: 728.

These authors describe glossitis, stomatitis, cheilitis and anorectal irritation as a complication of aureomycin (chlortetracycline) therapy.

53. Abbott, J. D., Fernando, A. V. J., Gurling, K. and Meade, B. N., 1952, Pulmonary aspergillosis following post-influenzal broncho-pneumonia treated with antibiotics. *British Medical Journal* **1**: 523.

A case report of pulmonary aspergillosis which appeared during antibiotic therapy for influenza-bronchopneumonia.

54. Brown, C. Jr., Propp, S., Guest, C. M., Beebe, R. T. and Early, L., 1953, Fatal fungus infection complicating antibiotic therapy. *J. A. M. A.* **152**: 206.

The authors describe five fatal cases of fulminating infection with *Candida albicans* occurring during antibiotic treatment with penicillin, chlortetracycline, and chloramphenicol.

55. Woods, James D., Manning, Isaac H. Jr. and Patterson, Carl N., 1951, Monilial infections complicating the therapeutic use of antibiotics. *J. A. M. A.* **145**: 207.

A report of 25 cases of moniliasis confirmed by culture occurring during antibiotic therapy. Twenty were cases of monilial infections of the oropharynx resulting from therapy with penicillin, chlortetracycline, or chloramphenicol. Three cases of monilial enteritis, one resulting from penicillin therapy, and two from therapy with chlortetracycline. Two cases of bronchopulmonary moniliasis resulting from treatment with penicillin and chlortetracycline.

56. Robinson, Harry M. Jr., 1954, Moniliasis complicating antibiotic therapy. *A. M. A. Archives of Dermatology and Syphilology* **70**: 640.

This author points out that monilia (*Candida albicans*) is present in normal human flora in 14 to 16% of normal persons, as determined by cultures from the oropharynx, the stools and the vagina. *In vitro* studies of stimulation of monilial growth by antibiotics are discussed. The author reports seven well-studied cases of moniliasis occurring during antibiotic therapy, and concludes that recovery of monilia alone is not sufficient to establish a diagnosis of moniliasis in all such cases seen.

57. Sharp, Jesse L., 1954, The growth of *Candida albicans* during antibiotic therapy. *Lancet* **1**: 390.

This author studied the incidence of *Candida albicans* in 174 patients admitted for treatment of pneumonia. The patients were divided into two groups, one group being treated with sulfadiazine (mean total dose 37 g) and the other group being treated with oxytetracycline (mean total dose 14.2 g). In the sulfadiazine group there was no increase in *Candida albicans* incidence during therapy. In the oxytetracycline group there was a marked increase in the incidence of *Candida* during treatment. None of the oxytetracycline group developed harmful effects from the increased incidence of *Candida albicans*.

58. Yow, Ellard M., 1952, Development of proteus and pseudomonas infections during antibiotic therapy. *Journal of the American Medical Association* **149**: 1184.

The author reports 56 patients with proteus and pseudomonas super infections occurring during therapy with penicillin, dihydrostreptomycin, chlortetracycline, chloramphenicol, streptomycin, sulfadiazine and sulfisoxazole. Selected cases are discussed in detail and therapy in each instance is described.

59. Finland, Maxwell., 1955, Changing patterns of resistance of certain common pathogenic bacteria to antimicrobial agents. *New England Journal of Medicine* **252**: 570.

Dr. Finland discusses the changing patterns of resistance of certain common human pathogenic bacteria to various antibiotics. He con-

cludes that the number of resistant strains of *Staphylococcus aureus* and the degree of resistance to penicillin and to a lesser extent to chlortetracycline and to oxytetracycline have shown a definite increase. No significant changes in resistance have occurred for pneumococcus, group A hemolytic streptococcus, meningococcus, gonococcus, *H. influenza* and *proteus*. A definite increase in resistance of pseudomonas strains to streptomycin and the broad spectrum antibiotics has occurred.

60. Sanford, Jay P., Favour, Cotting B. and Mao, Frances H., 1955, The emergence of antibiotic resistant gram-negative bacilli. *Journal of Laboratory and Clinical Medicine* 45: 540.

These authors studied the *in vitro* resistance of gram-negative human urinary tract pathogens. They compared the antibiotic resistance patterns of organisms recovered from patients before the "antibiotic era" with those isolated from patients hospitalized at the time of their study (1955). They concluded that the number of drug-resistant strains of bacteria causing urinary tract infections had significantly increased. *Escherichia coli* shows a definite increase in streptomycin resistance. Strains of *Pseudomonas aeruginosa* have developed marked resistance to streptomycin. An increase in streptomycin resistance by *Klebsiella pneumoniae*, *Aerobacter cloacae*, *Escherichia intermedium*, *Paracolon bacillus* and *Alcaligenes faecalis* was also demonstrated.

61. Eagle, Harry, Fleischman, Ralph and Levy, Mina, 1952, Development of increased bacterial resistance to antibiotics. Continuous spectrum of resistance to penicillin, Chloramphenicol and streptomycin. *Journal of Bacteriology* 63: 623.

Six bacterial species were studied, a type 3 group A β -hemolytic streptococcus, a group B β -hemolytic streptococcus, *Streptococcus faecalis*, *Diplococcus pneumoniae*, type III, *Micrococcus pyogenes* var. *aureus*, and *Escherichia coli*. The antibiotics used were sodium penicillin G, streptomycin sulfate and chloramphenicol. In a typical experiment with group A streptococcus in penicillin, the percentage of organisms growing out at concentrations of 0.004, 0.005, 0.006, 0.007 and 0.0085 micrograms per ml. was 86, 52, 0.25, 0.003 and 0.00003 respectively. Similar relationships were observed in many experiments with the other organisms and antibiotics. The individual organisms in the resistant cultures showed the same variation in resistance as the parent strain.

It is shown by these studies that the resistance of a colony which grows out in an antibiotic is generally related to the concentration of the antibiotic to which the organisms have been exposed.

62. Spink, Wesley W., 1954, Staphylococcal infections and the problem of antibiotic resistant staphylococci. A. M. A. *Archives of Internal Medicine* 94: 167.

Information available shows that the rising incidence of staphylococcal disease in hospitals is due to the appearance of strains of staphylococcus resistant to the antibiotics in common use. Dr. Spink discusses the four possible mechanisms to explain the appearance of resistant microorganisms. Typical clinical cases of resistant staphylococcal infections are discussed, with emphasis on early diagnosis and proper treatment.

63. Finland, Maxwell and Haight, Thomas H. with the Technical Assistance of Clare Wilcox and Marilyn K. Broderick, 1953, Antibiotic resistance of pathogenic staphylococci. Study of five hundred strains isolated at Boston City Hospital from October, 1951 to February, 1952. *A. M. A. Archives of Internal Medicine* **91**: 143, February.

Five hundred strains of hemolytic coagulase-positive *Staphylococcus aureus* were tested for sensitivity to nine antibiotics. The following pertinent conclusions emerged from these studies:

- 75% of strains were resistant to penicillin.
- 25% of strains were resistant to chlortetracycline.
- 50% of strains were sensitive to oxytetracycline, while about one-third were markedly resistant to oxytetracycline.
- 10% of strains were markedly resistant to streptomycin.

Resistance data to antibiotic combinations are presented and discussed.

64. Rountree, Phyllis and Thomson, Edgar F., 1952, Incidence of antibiotic-resistant staphylococci in a hospital. *Lancet* **2**: 262.

The monthly incidence of antibiotic-resistant strains isolated from 915 inpatients between January 1, 1951 and February 1, 1952 is reported together with figures for 157 outpatient strains isolated in the same period. These studies revealed an increasing incidence of penicillin resistance, streptomycin resistance and chlortetracycline-oxytetracycline resistance. These increases correlated with an increasing nasal carrier rate of antibiotic-resistant strains of staphylococci isolated from hospital personnel.

65. Knight, T. V. and Holzer, A. R., 1954, Studies on staphylococci from hospital patients. I. Predominance of strains of group III phage patterns which are resistant to multiple antibiotics. *J. Clin. Investigation* **33**: 1190-1198.

In a study of 516 strains of *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) principally cultured from the nose, throat and rectum of staphylococcal carriers at Bellevue Hospital in 1953 and 1954, it was found that 65% were resistant to chlortetracycline, oxytetracycline, streptomycin and penicillin. More than 90% of these drug-resistant strains were of phage group III patterns.

In content, 55 similar strains collected from 1932 to 1938 were susceptible to penicillin and were inhibited by low levels of chlortetracycline and oxytetracycline.

66. Goldberg, Herbert S. and Masterson, Byron J., Antibiotic sensitivity of *Micrococcus pyogenes* isolated from nursing students before and during hospital contact. *Antibiotics Annual 1956-1957*. Medical Encyclopedia, Inc.

Authors Summary:

"In the study of the staphylococcal carrier state in nursing students, 200 nasal cultures were isolated over an eight-month period. Analysis revealed that carriers of penicillin-resistant cultures increased from less than 10% to approximately 50%, carriers of coagulase-positive cultures from 8 to 45%, within a month of assuming hospital duties.

75. Goldberg, H. S., Goodman, R. N. and Lanning, Barbara, Low level, long term feeding of chlortetracycline and the emergence of antibiotic resistant enteric bacteria. *Antibiotics Annual 1958-1959*. Medical Encyclopedia Inc. (in Press).

On the basis of a 2-year study on feeding low-level (20-40 mg/animal/day) long-term antibiotics (streptomycin, chlortetracycline) to animals, in water and as residues in treated food, the following conclusions relative to emergence of resistance of intestinal flora in white Swiss mice can be made:

1. Five to forty per cent of all coliforms emerge resistant to levels of 10,000 mg/ml following streptomycin at long-term low-level feeding. Non-coliform lactose-negative organisms do not develop this resistance pattern.

2. Chlortetracycline does not cause detectable emergence of resistance by coliforms. Some resistance does occur with lactose-negative enterics (proteus-pseudomonas). However, these levels of resistance are minimal, and percentage of organisms exhibiting resistance is low when compared to the streptomycin results.

3. Long-term low-level feeding of chlortetracycline does not result in antibiotic blood serum levels which approach therapeutic levels. Daily feeding of low levels of chlortetracycline in food for 12 weeks resulted in antibiotic serum levels that were 1/100 of those attained by corresponding therapeutic doses.

4. It would seem that the emergence of antibiotic resistance in intestinal flora is less likely with tetracycline antibiotics than with streptomycin.

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